The Encapsulation and Release of Flutamide Using Poly (ε-Caprolactone) Microspheres

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STATEMENT OF ORIGINAL AUTHORSHIP

The work contained within this dissertation has not been previously submitted for a degree at any other higher institution. To the best of my knowledge and belief, the dissertation contains no material previously published or written by another person except where due reference is made.

Name : ……..

Signature : ……

Date : ……

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Abstract

In 2012 prostate cancer contributed towards 8% (1.1 million cases) of all cancer incidences around the world. This type of cancer is prevalent in men between the ages of 65-79 years old, with 25% of all cases occurring in men younger than 65 years old. Treatments that are currently available for prostate cancer include surgery, hormone therapy, radiation therapy and chemotherapy. These treatment methods are either very invasive or have harsh side effects including diarrhoea, nausea, alter liver function, anaemia and fatigue.

A wide range of anti-cancer drugs in use today have very poor physiochemical properties. New knowledge in this area is required to develop an advanced drug delivery system that improves the properties of these drugs. An example is anti-androgenic drugs such as flutamide (FLT) used in hormonal therapy. The disadvantages to FLT are that it has low bioavailability in oral formulations, low aqueous solubility, compliance issues and rapid first pass metabolism.

Recent advances in novel drug delivery have led to the formation of controlled release delivery systems using non-toxic polymeric microspheres. These polymeric microspheres encapsulate the active agent improving its bioavailability and compliance, reducing drug toxicity and side effects. The aim of this investigation was to develop a controlled release FLT delivery system in the form of poly (ε-caprolactone) (PCL) microspheres. The study was set out to evaluate the microspheres aesthetics, physicochemical properties and drug release behaviour. A central composite experimental design was employed to evaluate the effect of two process variables, (1) the polymer PCL at three different molecular weights (MW) 80kDa, 65kDa and 10kDa, (2) the surfactant (poly(vinyl alcohol) (PVA) at two molecular weight ranges 13-23kDa and 30-70kDa.

Preparing the organic phase consisted of 500mg of PCL and 50mg of FLT being completed dissolved in 10mL chloroform. The inorganic phase was formed by dissolving PVA in deionised water at a 0.5% weight/volume solution. The organic phase was added drop wise into the inorganic phase to create a 1.30 oil/water ratio. The emulsion was homogenised at 5000rpm for 1 minute. The chloroform was rotary evaporated off, followed by centrifugation and being frozen for 24 hours. The scanning electron microscopy (SEM) analysis was carried out with a freeze dried sample of the microspheres. The percentage yield was calculated to see how the sample amount changed with two process variables. Using laser diffraction, the average diameter of microspheres was determined. The percentage encapsulation efficiency (%EE) was carried out by dissolving PCL-FLT microspheres in

chloroform and ethanol. The solution was centrifuged and the UV-absorbance was recorded at 300nm. The *in-vitro* drug release was analysed via dissolution, PCL-FLT microspheres were suspended in a dialysis bag and stirred at 100rpm, in a phosphate buffer saline (PBS) solution.

The SEM data suggested the PCL 80kDa/ PVA 30-70kDa formulation produced the smoothest and most uniform microspheres with the highest mean percentage encapsulation efficiency at 90.92% ±1.08%. The micrographs showed that as the PCL MW increased from 10kDa to 80kDa the particle size increased from 5.5µm to 8.4µm. Regarding percentage yield the 80kDa/ PVA 13-23kDa FLT loaded formulations produced the most amount of product, averaging at 72.95% ±1.28%. However, after statistical analysis of %EE and product yield there was no significant difference in data between the two MW ranges of PVA ($P>0.05$). Dissolution results showed PCL 80kDa/ PVA 30-70kDa microspheres to have a maximal release of 80.23% over 16 days with an intial burst release of 15.38% within the first 4 hours of dissolution. This suggested that encapsulted FLT microspheres can be administered less frequently (once every 2 weeks) at a lower dose (50mg), as the release rate (80.23%/ 16 days) of encapsulted FLT is slower than the half life of free FLT (8 hours). Overall the formulation that produced the most ideal microspheres regarding aesthetics, size, yield, encapsulation efficiency and dissolution was the PCL 80kDa/ PVA 30-70kDa formulation.

Further studies that can be conducted include transition electron microscopy (TEM) analysis to evaluate the internal components of the PCL-FLT microsphere complex. A co-polymer such as poly(lactic-co-glycolic acid) (PLGA) can be incorparated along side PCL in order to further improve the encapsulation efficiency. Toxicity studies can also be carried out involving prostate cancer cell lines (MTT Assay).

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1.0. Introduction

1.1. Background of Cancer

Worldwide cancer is assessed as an enormous burden that is expected to increase due to the growth and aging of the population. Lifestyle behaviours also influence the risk of developing cancer, such as smoking, poor diet and physical inactivity. The focus of this study is prostate cancer which in 2012 contributed to 8% of all cancer incidences at 1.1 million cases worldwide (Ferlay *et al*., 2012).

The most common type of cancer found in men is prostate cancer, and makes up 26% of all male cancer diagnosis in the UK. In 2008, there were 34,335 men diagnosed with prostate cancer and 9376 deaths as a result of the disease in England, Wales and Northern Ireland. In 2010 the figures increased to 9632 deaths. This type of cancer is predominant in men aged 65-79 years but, about 25% of all cases occur in men younger than 65 years old (Cancer of the Prostate, 2011).

1.2. About Prostate Cancer/ Current Therapies

Prostate cancer is a disease of the prostate gland. The gland is approximately the same size as a walnut, present only in men in the pelvic region surrounding the urethra. The function of the prostate gland is to secrete the liquid portion of male semen, or seminal fluid which then carries the sperm made by the testes (seminal fluid is essential for reproduction) (Cramer *et al*., 2007; Kumar and Majumder, 1997).

The size of the prostate gland can increases with age. This is not a result of cancer but is a condition known as benign prostatic hyperplasia (BPH). BPH does not usually form into cancer but an enlarged prostate gland can occasionally contain areas of cancerous cells. There are no specific symptoms for early prostate cancer.

The majority of prostate cancers initially form on the outer part of the prostate gland, away from the urethra. If the tumour is not large enough to put pressure on the urethra that transports urine out of the body, there may not be any symptoms that arise (Balistreri *et al*., 2014; Francini *et al*., 2014).

As the benign or cancerous tumor grows there are a number of symptoms that may occur which include;

- Difficulty passing urine, straining to pass it or stopping and starting
- Rushing to the toilet (uncontrollable urination)
- Increased frequency of passing urine (commonly at night)
- A sense of not being able to fully empty the bladder
- Blood when passing semen or urine (rare)
- Pain during urination (rare symptom)

Current Therapies

The most common treatments for prostate cancer are surgery, hormone therapy, radiation therapy and chemotherapy (Balistreri *et al*., 2014; Francini *et al*., 2014).

Surgery

The type of surgery depends on;

- The size of the cancer and whether it has spread
- What the cells look like under the microscope (*Gleason score)
- General health

*****Gleason score: A Gleason score is given to prostate cancer based upon its microscopic appearance. Cancers with a higher Gleason score (5-7) or (8-10) are more aggressive and have a worse prognosis (Gleason and Mellinger, 1974).

A treatment option known as a total prostatectomy or radical prostatectomy which is the removal of the prostate gland may be recommended. This surgical procedure may often cure the prostate cancer if it has not spread beyond the prostate gland. Although this treatment can cure the cancer there may be some long term side effects such as erection problems which occurs in 70% of all patients. Another side effect is urine leakage, making it difficult to control the flow of urine. This is due to muscle damage within the bladder, and can be seen in 20% of all patients (Waxman *et al*., 1997).

Another option is an orchidectomy where the individual has both testicles removed. This procedure is carried out so the body cannot produce the hormone testosterone anymore. As prostate cancer is testosterone dependant, by removing the hormone producing gland it can shrink a locally advanced cancer or stop it from growing. Removing the testicles does not cure the cancer but can be used to control the growth of the cancer for months or even years. After surgery some men experience side effects such as hot flushes or/and erection problems. A less invasive method to prevent testosterone from being made is hormonal treatment (Paula *et al.*, 2003).

A surgery known as transurethral resection (TUR) can be recommended which involves the removal of the inner part of the prostate gland from around the urethra. This operation relieves symptoms such as being unable to pass urine. Having a TUR done is not a cure for prostate cancer but just a temporary symptom reliever (Marszalek *et al*., 2009).

Radiotherapy

External radiotherapy involves high doses of radiation which focuses on the prostate gland area. The treatment ensures the whole of the prostate gland is treated to a surround area of 1-2cm around the cancerous cells. This is to make sure that any cancerous cells that are close to the tumor are also treated (microscopic spread).

A patient can have external radiotherapy treatment if the cancer is between stage T1 and T3. This means the cancer is too small to be seen on scans or felt during an examination of the prostate gland, to the cancer covering the entire prostate gland and no further. If it has spread to different organs this treatment is likely to be ineffective. Usually treatment is given once a day, for 5 days each week. This course of administration normally lasts between 4-8 weeks (Greene *et al*., 2002).

Internal radiotherapy in another option that involves that involves the inner part of the prostate gland. This is sometimes a curative method when the prostate cancer is completely contained within the prostate. This treatment is known as a brachytherapy and is normally recommended when the cancer is low risk. There are two types of brachytherapy, one being seed implantation low dose rate therapy (LDR) and the other being radioactive tube implantation - high dose rate therapy (HDR). The preferred choice with some patients is brachytherapy as they only need to attend the hospital once or twice.

Brachytherapy may come with side effects post-surgery. The most common side effects are difficulty passing urine. Around 15% of men will not be able to voluntarily pass urine and a catheter is passed into the bladder for a few days (Cooperberg *et al*., 2010).

Hormone Therapy

The function of hormones is to control the growth and activity of normal cells. Prostate cancer cells depend on the testosterone to proliferate. Hormone treatment can be used to lower the amount of testosterone in the body, which reduces the risk of an early prostate cancer returning back after treatment or shrink an advanced tumor to slow its growth (Brawer, 2006). Depending on what stage the cancer has reached, hormone treatment can be considered as a monotherapy or in conjunction with chemotherapy.

Anti-androgenic drugs such as bicalutamide (Casodex), flutamide (Drogenil or Eulexin) or enzalutamide (Xtandi) can be prescribed. One of the most common hormonal treatments is flutamide. flutamide prevents testosterone initiating cellular division and growth which intern slows down the cancerous growth or shrinks an advanced tumor (Debruyna, 1996). It has been seen that between 1 and 10 patients suffer from the following side effects;

- Changes in liver functionality (during treatment)
- Mild case of feeling or being sick
- Mild case of diarrhoea
- Tiredness & fatigue
- Emotional Distress
- Difficulty sleeping
- Reduced red blood cell count (anaemia)

In conjunction with flutamide (FLT), the individual may be prescribed luteinising hormone (LH) blockers. This will prevent the pituitary gland from releasing the hormone that signals the testicles to produce testosterone (Horwich, 2006). Examples of LH blockers are:

- Leuprorelin (Prostap)
- Goserelin acetate (Zoladex)
- Buserelin (Suprefact)
- Triptorelin (Decapeptyl)
- Histrelin (Vantas)

These drugs are commonly administered intravenously, some being given every month, and others every 6 months (Horwich, 2006).

Chemotherapy

Chemotherapy involves killing cancer cells using drugs. This method is only used if the cancer has spread to other body parts. In regards to prostate cancer the most common drug used in chemotherapy is Docetaxel (Taxotere). It may be used as a first line treatment alongside hormone therapy such as FLT if the prostate cancer has spread. Chemotherapy is quite an effective treatment over a long time period, but comes with a series of side effects depending on the drug combination. The most common side effects are a compromised immune system making the patient susceptible to infection, tiredness and fatigue and being prone to nose bleed and other bleeding issues (Beekman *et al*., 2005).

1.3. Controlled Drug Delivery

Current drug carrier systems, i.e. injectable implants, play a key role in controlled delivery of pharmacological drugs. Parenteral controlled release systems are of considerable importance for drugs as they require daily administration or have low bioavailability or high toxicity (Sandrap and Moes, 1993).

When looking at the current administration of the anti-androgenic drug FLT (250mg) during the treatment it produces high levels of drug toxicity in the blood which can lead to long term side effects. The major side effect that continues after treatment has finished is loss of bone density and bone strength. It would be ideal if these drugs were made available in the blood at effective concentrations with reduced side effects for a longer duration. A way around the daily administration of drugs is the usage of polymeric carriers (nanospheres and microspheres) as controlled release systems (Blanco-Prieto *et al*., 1997).

Producing biodegradable injectable delivery systems to transport drugs and to preserve the blood level in a desired therapeutic range for a long period of time improves compliance.

The design of the controlled release dosage form has other advantages over conventional dosage forms (Fell, 1996; Baumgartner *et al*., 2000; Moës, 1993):

- Reduction in frequency of drug administration.
- Reduction in drug level fluctuation.
- Reduction in total drug used in comparison to conventional therapy.
- Reduction in drug toxicity (Local/ Systemic).
- Stabilisation of medical condition due to more uniform drug levels.
- Economical to health care providers and patients.

1.4. Biodegradable Polymers in Controlled Drug Delivery.

In the pharmaceutical industry there are a variety of different polymers that are being used for controlled drug delivery. They can be synthetically manipulated for the use of many applications such as biofilms, nano-particles and micro-particles. Common polymers that are used include Poly-D,L-lactide-co-glycolide (PLGA), Polylactic Acid (PLA) or poly(ε-caprolactone) (PCL).

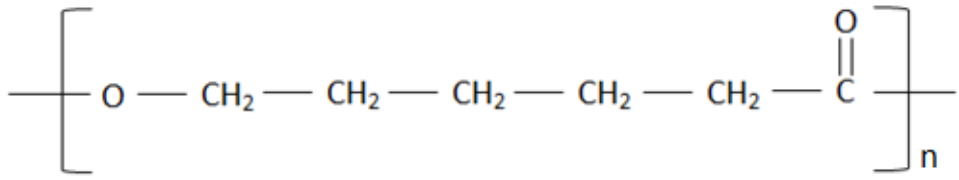
PLGA is known be a successfully used biodegradable microsystem for drug delivery as it undergoes hydrolysis in the body to produce biodegradable metabolite monomers such as lactic acid and glycolic acid. The body can effectively deal with these two monomers by metabolising them within the TCA cycle. PLGA is FDA approved for human use and has already been used to incorporate cancer therapeutic drugs such as Taxol, Paclitaxel and Docetaxel (Sahana *et al*., 2008).

PLA is also a biodegradable and biocompatible material that undergoes scission into monomeric units of lactic acid as a natural intermediate in carbohydrate metabolism. PLA nano and microparticles are prepared using a technique known as solvent evaporation. This encapsulation method delivers the least amount of stress to the drug or proteins encapsulated. This polymer has

been used to encapsulate proteins such as haemoglobin, protein-c and neurotoxin-i (Fessi *et al*., 1989).

1.5. Poly(Ɛ-Caprolactone)

The synthetic polymer PCL (figure 1) has been of particular interest as it allows for a long sustained and possible controlled drug release rate (Chawla and Amiji, 2002). It has a very low glass transition temperature of approximately -60^{o}C and has a melting point ranging between 59-64^{o}C depending on chain length (molecular weight). PCL is ideal for controlled drug delivery in targeted cancer therapy, as it has a high permeability to a wide range of drugs and is non-toxic (Murthy, 1997). PCL is a biocompatible polymer with a very slow degradation rate, this makes it very ideal for long term delivery. As it is a semi crystalline synthetic polymer and is extensively used in the pharmaceutical and biomedical fields as a biomaterial and for prolonged drug delivery systems targeting specific tissues within the body (Sinha *et al*., 2004). Out of the three polymers (PLGA, PLA and PCL), PCL is the most economically viable option being the cheapest to produce and has the least toxicity within the body. Also unlike PLA and PLG polymers PCL does not generate an acid environment, which could adversely affect the stability of a drug. Currently hormone altering drugs (FLT) have not been encapsulated within PCL microparticles. These types of drugs would be ideal candidates to be encapsulated within a PCL microsphere to initiate controlled release of the active agent. The polymer PCL undergoes hydrolysis of the ester linkages within the human body and has a slower degradation rate compared to the other lactide derived polymers (Kumari *et al*., 2010).



Molecular weight: $(114.14\ g/mol)_n$

Figure 1. Chemical structure of a free Poly(Ɛ-Caprolactone) monomer unit (Created using Microsoft Word. 2010).

1.6. Chloroform (Solvent)

The polymer PCL during the solvent evaporation process requires an appropriate solvent to dissolve the bioactive agent (Anti-cancer drug) and PCL polymer. Table 1 below shows a variety of the volatile solvents that are suitable and not suitable for dissolving PCL. Chloroform is an ideal solvent as it has the following characteristics; low boiling point (61^{o}C), high volatility as well as being good at dissolving PCL (Maia *et al*., 2004). The bio-active agent FLT is also soluble in chloroform (Sigma Aldrich).

During the solvent evaporation step in figure 4 the polymer and the solvent properties have to be taken into account to prevent any damage to the sample. The PCL has a boiling point melting ranging between 59^{o}C and 64^{o}C and has a polymer softening temperature of 35^{o}C as listed by Sigma-Aldrich. The solvent Chloroform has a boiling point of 61^{o}C which suggests for the microspheres to retain their rigidity during this process the rotary evaporator will be set at a constant controlled temperature of 30^{o}C.

Table 1. Solvents used for PCL polymer solubility (Chang *et al*., 1986).

Poly-ε-caprolactone (PCL) Solvent Solubility		
(High Solubility)	(Low Solubility)	(Insoluble in)
Chloroform	Acetone	Alcohol
Dichloromethane	2-butanone	Petroleum Ether
Carbon tetrachloride	Ethyl-acetate	Di-ethyl ether
Benzene	Dimethylformamide	
Toluene	Acetonitrile	
Cyclohexane		
2-nitropropane		

1.7. Flutamide

The therapeutic drug involved in this investigation is flutamide (FLT) also known as Eulexin or Drogenil with an average molecular weight of 276.2 g/mol (Sortino *et al*., 2001). FLT shown in figure 2 is prescribed as a hormonal therapeutic drug in combination with a luteinising hormone (LH)

blockers for treating prostate cancer (Martel *et al*., 2003). FLT is an anti-androgenic agent that is therapeutically effective for benign prostatic hypertrophy (BPH) and androgen dependent prostate cancer (Debruyna, 1996).

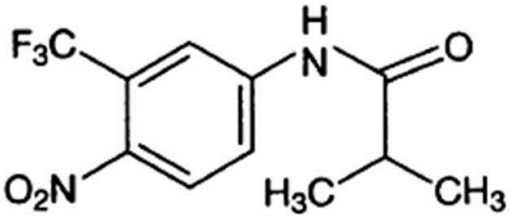
Conventionally FLT 250mg (or 500mg) is orally administered three times daily which is in accordance to Cancer Research UK. The regular administration of FLT has a half-life of approximately 8 hours with 10 metabolites (Lemke and Williams, 2013). After oral administration FLT is absorbed from the gastrointestinal tract with a t_{max} of about 2 h. The two major metabolites after first pass metabolism are 2-hydroxyflutamide and the hydrolysis product 3-trifluromethyl-4-nitroalanine. Schulz and his team of researchers in 1988 saw that after a single 250mg dose of FLT the average maximum FLT plasma concentrations is 0.02 $\mu g \cdot ml^{-1}$ (n=3) (Schulz *et al*., 1988).

The steroid hormones known as testosterone (androgens) have aids the proliferation of prostate tumors (Huggins and Hodges, 1941). Therapeutic agents such as anti-androgen are of great importance to society as they reduce androgen levels in target tissues. Two of the most common drugs currently being prescribed to treat prostate cancer are finasteride (Marketed by Merck & Co. as Proscar) and flutamide (Marketed by Schering-Plough as Eulexin).

The drug FLT has a low bioavailability in oral formulations, which may be due to the poor wettability, low aqueous solubility (0.00566 mg/mL), low concentration at the absorption surface and rapid first pass hepatic metabolism after oral administration (Nari, 1989).

It is necessary to develop an innovative formulation that mitigate solubility and dissolution sustaining a higher concentration of FLT at the absorption site, by overcoming first pass metabolism. The dosage characteristics of FLT (250mg three times daily) have indicated that it is a suitable candidate for a controlled-release delivery system, to improve patient's compliance and reduce the

occurrence of side effects (Zuo *et al*., 2002). Despite these statistics, to our knowledge there has been no controlled release formulation of FLT that has been prepared until now (Elgindy *et al*., 2010). The bio-active agent FLT shown in figure 2 is soluble in the solvent chloroform which will be used primarily in this investigation (Sigma Aldrich).



Molecular weight: (276.21 g/mol)

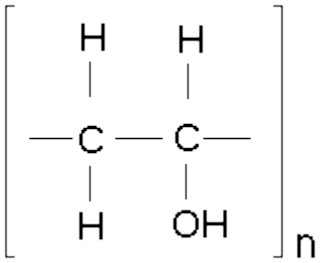
Figure 2. Molecular structure of free flutamide (2-methyl-N-[4-nitro-3 (trifluoromethyl)phenyl]propanamide) (Sortino *et al*., 2001).

In this investigation FLT is being encapsulated with in a PCL polymer. To test for the presence of FLT throughout this study during various experiments the UV detection wavelength is 296nm, which detects the b-band of the benzene ring in the structure. This wavelength is in accordance to Filip, M. and his team of researchers in 2007 regarding the "HPLC Monitoring of FLT Drug Used in the Prostate Cancer Treatment" (Filip *et al*., 2007). This wave length of 296 nm will be verified further in this current study.

1.8. Surfactant

During the formulation process a variety of excipients can be added to improve the stability of the drug during fabrication. The polymer-solvent-drug solution is emulsified by poly(vinyl alcohol) (PVA). The PVA shown in figure 3 is a white non-toxic biodegradable semi-crystalline polymer that can be used as an emulsifier or surfactant. It also has a very good aqueous solubility and is incorporated in the water phase of the preparation (Yang *et al*., 2001).

The reasoning for using a surfactant such as PVA during the production of polymeric microspheres is to reduce the amount of microsphere clumping producing a more uniform product (Arshady, 1991). Another reason for including a surfactant such as PVA is to stabilise the primary emulsion, resulting in more uniform distribution of microspheres (Yang *et al*., 2001). PVA will be tested as a surfactant in this investigation at two different molecular weight ranges (13-23kDa or 30-70kDa).



Molecular weight: $(44.05\ g/mol)_n$

Figure 3. Molecular structure of a Poly(Vinyl Alcohol) monomer unit (Rowe, 2012).

1.9. Release Studies

The fundamentals of controlled drug release is to change the pharmacokinetics and pharmacodynamics of a pharmacologically active agents by using advanced drug delivery systems, or altering the molecular structure and/ or physiological parameters occurring within a selected route of administration. During the controlled release of the polymer-drug microspheres they incorporate The controlled release delivery system encapsulates a drug within a polymeric microsphere the active agent is either dispersed or dissolved throughout the particle matrix (Neetika *et al*., 2012).

The release kinetics of microspheres drug delivery systems suggest that if the concentration of drug encapsulated in the polymer core is constant (dependant on the drugs solubility in the solvent), the driving force of the drug release is constantly diffusing through the polymer membrane. The factors that influence the release rate are release area, thickness of the polymeric membrane, the implant form (microspheres) and drug solubility (Bourges *et al*., 2006).

Other dependant factors that can affect drug release are rate of diffusion of the drug from the delivery system, erosion of the polymer matric and biodegradation of the polymer. In cases where biodegradation and erosion of the polymer are slow, the release rate is strongly influenced by the drug diffusion pathway. The interaction between the drug-polymer, the pKA of the drug, the amount of drug loaded in the microsphere and the state of incorporation of the drug in the delivery system can influence the diffusion rate. The state of incorporation of the drug in the system and interaction between drug-polymer are important factors that affect the release profile (Maysinger *et al*., 2001).

1.10. Research Aim

In this study, it was aimed to develop a controlled release FLT delivery system in the form of PCL microspheres and investigate it for physicochemical properties and drug release behaviour. A central composite experimental design was employed to evaluate the effect of two process variables: **i)** The polymer poly (ε-caprolactone), three PCL molecular weights (80, 65 and 10kDa). **ii)** The surfactant (poly(vinyl alcohol), two PVA molecular weight ranges (13-23kDa and 30-70kDa). The objectives of the present study are to:

- Prepare 12 formulations of PCL microspheres.
    - 6 empty PCL microspheres (using the two process variables mentioned above).
    - 6 FLT loaded PCL microspheres (using the two process variables mentioned above).
- To determine the effects of different PVA molecular weight ranges via SEM analysis.
- To investigate the effects of different PCL molecular weights on particle size.
- To analyse the effects of different PCL molecular weights on encapsulation efficiency (Direct Method).
- To analyse the effects of different PCL molecular weights on product yield.
- To investigate the factors that influence different release profiles.
- To determine the effect of different PCL molecular weights on the rate of drug release.

Taking all these objectives into account an overall conclusion can be deduced regarding which formulations are the most ideal for further development.

2.0. Materials and Methodology

2.1. Materials

Poly-Ɛ-Caprolactone (PCL) ($C_6H_{10}O_2$, 80,000 MW; CAS-No 24980-41-4)/ ($C_6H_{10}O_2$, 65,000 MW)/ ($C_6H_{10}O_2$, 10,000 MW), Flutamide (FLT) 2-methyl-N-[4-nitro-3 (trifluoromethyl) phenyl] propanamide (CAS-No. 13311-84-7), Chloroform (99%) ($CHCl_3$, CAS-No. 67-66-3) and Ethanol (99%) (C_2H_6O, CAS-No. 64-17-5) were all purchased from Sigma-Aldrich (Sigma-Aldrich inc, 3050 Spruce St., St. Louis, MO63103). Poly Vinyl Alcohol (13-23 and 30-70kDa) was purchased from Fluka Analytical. Phosphate Buffer Saline (Dulbecco A) was purchased from Oxoid Microbiology. Dialysis Membrane bags (Pur-A-Lyzer™ Mega Dialysis Kit - 3-20ml - 3.5kDa MW - CAS-No. PURG35020-1KT) were purchased from Sigma-Aldrich (Sigma-Aldrich inc, 3050 Spruce St., St. Louis, MO63103).

2.1.1. Equipment

Homogeniser (IKA Labortechnik ULTRATURRAX TP 18/10 – 1000 – 10000 rpm), Rotovap Büchi Rotovapor re120, Varian 705 DS Dissolution Apparatus, Mastersizer (Malvern Mastersizer 3000 laser diffraction particle size analyser), Zeiss EVO50 SEM operated at (EHT) 10.00 kV and UV-spectrophotometer, Perkin Elmer Lambda XLS (430nm), were all provided by the University of Wolverhampton.

2.2. Methods Development

Prior to starting this laboratory investigation independent research was carried out to further develop the current protocols used in the Tang lab.

The preparation process of microsphere particles is determined by the solubility of the drug and polymer (PCL) in various solvent systems. Two common systems used are;

- Single emulsion solvent evaporation (Perez *et al*., 2000).
- Double emulsion solvent evaporation (Dhanaraju *et al*., 2006; Cleek *et al*., 1997).

The most common method of producing PCL microspheres is the single emulsion oil/ water (O/W) solvent evaporation method (figure 4), which will be used to carry out this investigation. The diagram in figure 4 summarises the production of drug loaded polymeric microspheres. There are 4 distinctive steps involved (Li *et al*., 2008);

1. Dissolving of the synthetic polymer in an ideal volatile organic solvent, then the addition of the active compound (active compound can be dissolved or simply dispersed in the organic phase).

2. The emulsion of the organic phase is transferred in to an immiscible aqueous phase, forming the Oil/Water emulsion.

3. The removal of the solvent from the dispersed phase, via solvent evaporation. Transforming the dispersed phase into solid particles.

4. The harvesting and drying of the microspheres.

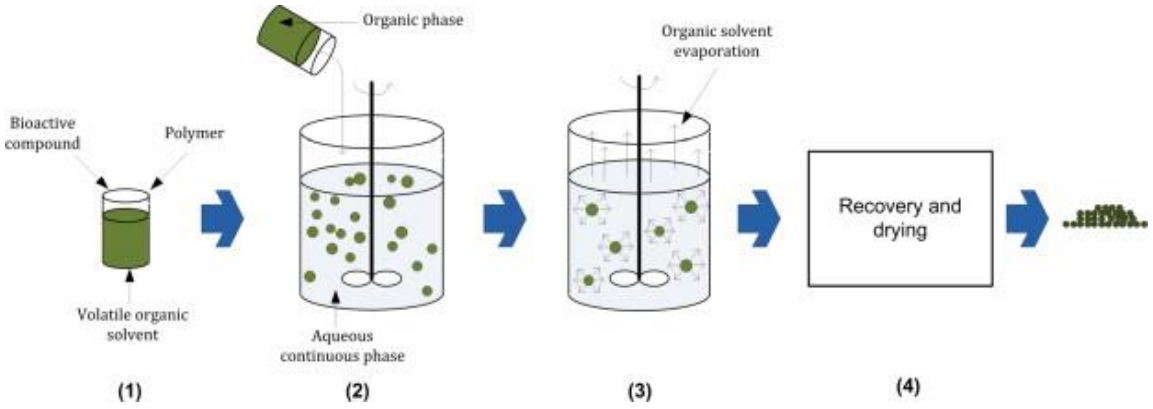


Figure 4. Main steps of the oil/water single emulsion-solvent evaporation method (Li *et al.*, 2008).

2.3. Investigation Outline

PCL microspheres were formulated using a protocol that was previously used within Professor James Tang's lab at the University of Wolverhampton. His past research suggested that microspheres made using PCL 80kDa produced the most ideal microspheres for the controlled drug release of FLT. However past investigation haven't looked into using low a PCL molecular weight such as PCL 10kDa and different (Surfactant) PVA molecular weight ranges.

This investigation analysed three different molecular weights of PCL microspheres (80kDa, 65kDa and 10kDa). Each microsphere formulation was created to also analyse the effects of two different PVA molecular weight ranges (13-23kDa and 30-70kDa). The microspheres formulations were prepared using a (single emulsion) solvent evaporation method. The investigation will include:

- SEM analysis.
- Percentage yield analysis (n=4).
- Particle size analysis (n=5).
- Percentage encapsulation efficiency analysis (n=3).
- Drug release profile testing (n=3).

This will suggest which formulation produces microspheres with optimal characteristics.

2.4. Preparing the Organic and Inorganic Phase

500mg of PCL (80kDa, 65kDa and 10kDa) and 50mg of FLT were completely dissolved in 10mL Chloroform to make up the oil phase (organic phase). Then 1.25g of PVA (13-23kDa or 30-70kDa) was completely dissolved into 100mL deionised water, making up the water (inorganic) phase. This was then filtered using a 0.45µm filter into a 250mL conical flask and made up to 0.5% (wt/v) concentration with deionised water. This was then repeated with each MW of PCL (80kDa, 65kDa and 10kDa) and both low and medium MW of PVA surfactant (13-23kDa and 30-70kDa respectively).

- When formulating the control microspheres (without FLT), during the preparation of the organic phase no FLT was added into the formulation.

2.5. Preparing Microspheres

2.5.1. Single Emulsion Drug Encapsulation

The microspheres were formed using a 1:30 oil/water ratio (Pilaniya *et al*., 2011). 8mL of the organic phase (Oil) was added to 240mL of the inorganic phase (Water). This was then homogenised for 1 minute at 5000 rpm using the IKA Labortechnik ULTRATURRAX TP 18/10.

2.5.2. Solvent Evaporation/ Centrifugation and Freeze Drying

The organic solvent (Chloroform) was then evaporated off at 30^{o}C using Büchi Rotovapor re120 for 20 - 30 minutes. The emulsion was then transferred to centrifuge tubes and centrifuged for 1 minute at 1000 x *g,* 1 minute at 3000 x *g* and 1 minute at 4500 x *g,* to turn the emulsion into a concentrated pellet of PCL-FLT microspheres. Upon centrifugation the samples were frozen for 24 hours (-18^{o}C) in preparation for the drying process. To remove the moisture and dry the sample it was then freeze dried using an Edwards EF4 Modulyo bench top freeze drier for 6 - 8 hours.

2.6. Particle Size Measurement

All formulations were assessed using a particle size mastersizer (Malvern Mastersizer 3000 laser diffraction particle size analyser). After the rotary evaporation step a 2ml sample of the emulsion was added drop wise to 100mL of deionised water until an obscuration rate of 15-30% was obtained. The sample was analysed for size distribution and particle size over five replicates. An absorption index of 0.001 and refractive index of 1.500 was used. The results were displayed as 10, 50 and 90% of the sample. After the initial analysis each sample was subjected to ultra-sonication to separate any large clumps of microspheres.

2.7. Scanning Electron Microscopy

Dry microspheres were placed onto an aluminium stub using double sided carbon tape and sputter coated with gold. They were placed into a Zeiss EVO50 scanning electron microscope, which is operated at (EHT) 10.00 kV. Various photomicrographs were taken at different magnifications for each sample.

2.8. *In-Vitro* Drug Release

In-vitro drug release of FLT from the drug loaded PCL microspheres was assesed using the dissolution tank method. The microspheres (20mg) were suspended in 20mL of phosphate buffer saline (PBS), pH 7.4, and placed into dialysis bags (Pur-A-Lyzer™ Mega Dialysis Kit - 3-20ml - 3,500kDa MW). The bags were tied to the paddle of the dissolution tank (Varian 705 DS Dissolution Apparatus) and dialysed against 900mL PBS, pH 7.4, as the dissolution medium. The dissolution system was maintained at 37 ^{o}C ± 0.5^{o}C and was constantly stirred at 100 rpm. The total amount of drug released at set time intervals was determined by removing 5mL of the release media and replenished with an

equal volume of fresh preheated (37^{o}C) PBS. All samples were centrifuged at 4500 x *g*, and analysed ultraviolet absorbance at 300nm wavelength to detect the b-band of the benzene ring in FLT (Perkin Elmer Lambda XLS) using PBS as the blank (Elzoghby *et al*., 2013).

- According to Philip, M and his team of researchers in 2007, they concluded that the b-band of benzene in FLT is detected at 296nm of ultraviolet light. To confirm this, a FLT stock solution (0.05mg/ml ethanol) was scanned in a 1.4mL quartz cuvette using the Perkin Elmer Lambda XLS to produce an absorbance profile. The aborbance profile peaked at exactly 300nm on the spectrophotometer suggesting that the b-band of the benzene ring in FLT is detected at 300nm.

2.9. Direct Encapsulation Efficiency

Each sample (5mg) was completely dissolved in 0.5mL Chloroform and diluted with 10mL Ethanol. The samples were then centrifuged for three minutes (1 min at 1000 x *g,* 1 min at 3000 x *g* and 1 min at 4500 x *g*). The sample solutions were then transferred into a 25mL volumetric flask and diluted with more Ethanol, the absorbance of each sample was then analysed UV-spectrophotometrically (Perkin Elmer Lambda XLS) 300nm (Sivabalan *et al*., 2012). Each sample was read and the carried out in triplicate.

To calculate the percentage efficiency will be converted from an arbitrary absorbance reading to a concentration using the line equation from the FLT calibration curve (dividing by the y= value). The formula (Chhater and Praveen, 2013):

$$\text{Percentage Encapsulation Efficiency} = \frac{Actual\ Drug\ Content\ (mg)}{Theoretical\ Drug\ Content\ (mg)} \times 100$$

2.10. Indirect Encapsulation Efficiency

The supernatant of each sample was collected after centrifugation during the production step (2.5.2.). Then 1mL of supernatant was extracted and re-centrifuged for 5 minutes at 5000 x *g*. 200µL of the supernatant was transferred into fresh Eppendorf tubes, into which 800µL of 95% ethanol was added to make up a 1mL solution. The samples were then thoroughly mixed and the absorbance's read spectrophotometrically at 300nm (Perkin Elmer Lambda XLS).

- Both the direct and indirect method can determine the percentage encapsulation of FLT. However the direct method is the most accurate technique to deduce the percentage encapsulation. The direct method breaks down the polymer capsule and detects the amount of FLT that is encapsulated within the microspheres to an accurate percentage. The only disadvantage to this method is that it will also take into account any unencapsulated FLT that is bound to the external surface of the microsphere. The indirect method detects encapsulation via calculating the amount of unencapsulated free FLT remaining in the supernatant after the rotary evaporation and centrifugation step. The encapsulated amount can be indirectly calculated from the absorbance readings (at 300nm). This method indirectly calculates the amount of FLT that should be encapsulated but can only give an approximate value as opposed to the direct method, which is the most common method used and more accurate.

2.11. Percentage Yield

The percentage yield was calculated to determine the total amount of product obtained from the raw materials. The total percentage yield was calculated using the following formula (Prabu *et al*., 2009):

$$\text{Percentage Yield} = \frac{total\ amount\ of\ microspheres\ obtained\ (dry\ weight)}{total\ amount\ polymer+drug} \times 100$$

- The surfactant (PVA) is not included within the percentage yield formula because after the microspheres emulsion has been centrifuged and separated from the supernatant, followed by freezing and freeze drying the moisture has been removed. Therefore the amount of PVA remaining is in the dry sample is considered negligible.

3.0. Results

3.1. Scanning Electron Microscopy

Figure 5 displays 12 comparative scanning electron microscope (SEM) micrographs (A-L), 6 of which show unencapsulated microspheres at 3 different poly (ε-caprolactone) (PCL) molecular weights (80kDa, 65kDa and 10kDa) and 2 different poly vinyl alcohol (PVA) molecular weight ranges (13-23kDa and 30-70kDa). The remaining 6 micrographs show FLT encapsulated microsphere at the same formulation variables as the control batches (See Appendix 3.1.1. for detailed formulations).

Image **A** illustrates fully formed smooth microspheres, with the majority being the same size showing an average diameter of 8.50µm diameter (n=10). It is apparent that there is low coagulation and all the chloroform (solvent) has been evaporated off efficiently, this is apparent from the spherical shape and the wrinkleless shell. Smaller 2.00µm (n=10) microspheres can be seen surrounding the larger microspheres (ratio: 1 Large : 2 small). When observing SEM image **B** there are two definitive sizes in this sample with little indication of uniformity. The majority of microspheres are showing an average diameter of 8.30µm (n=10). The micrograph also displays a large amount of small microspheres at an average 2.00µm in diameter (n=10) that have been formed surrounding the larger microspheres (ratio: 2 Large: 1 Small) (Scale Bars : 2.00µm).

Image **C** shows microspheres that are smooth and uniformly spherical at an average diameter of 10.00µm (n=9), with is no indication of clumping or major wrinkling. There is an indication of one to two 2.00µm microspheres of PCL that can be seen in between the microspheres. SEM image **D** shows microspheres at an average diameter of 8.40µm (n=10) that are smooth and uniformly spherical, with is no indication of clumping or major wrinkling (Scale Bars : 2.00µm).

Image **E** show fully formed microspheres at an average diameter of 8.30µm (n=3) with a minute amount of wrinkles on the surface of the spheres. Although fairly smooth, slight deformities are seen attached to the surface. These are clumps of PCL polymer about 1.00µm that haven't fully formed and attached to the outer surface of the microspheres during the formulation process. SEM image **F** shows smoothly formed microspheres at an average diameter of 7.20µm (n=4) that have very little wrinkles and have a lot of uniformity as the spheres are nearly all the same size. Small PCL clumps seem to be visible, but unlike image **E** the free PCL polymer has not hindered the spherical shape of the microspheres (Scale Bars : 2.00µm).

Image **G** shows fully formed microspheres at an average diameter of 9.60µm (n=3) that are quite smooth with a regular spherical shape and no clumping. The uneven layering seen on the background is due to both the structure of the carbon sticky, or scraped PCL polymer upon application. SEM image **H** show fully formed microspheres at an average diameter of 9.90µm (n=10) that have some wrinkles on the exterior. The majority of spheres are uniform in size with very little wrinkles, over all showing a fairly smooth exterior (Scale Bars: 2.00µm).

Image **I** shows fully formed microspheres at an average diameter of 6.10µm (n=3) that are quite smooth with no clumping and minimal reference to surface wrinkles. SEM image **J** show fully formed microspheres at an average diameter of 5.90µm (n=4) that have some wrinkles on the exterior. The spheres are reasonably uniform but throughout the sample uniformity isn't as apparent as sample **I** (Scale Bars : 2.00µm).

Image **K** illustrates fully formed smooth microspheres, with the majority being uniform in size. It is apparent that there is no coagulation between microspheres and structurally look spherical with a wrinkleless exterior. When observing SEM image **L** there are a variety of different sizes in this sample with minor indication of uniformity. The largest microspheres are at an average diameter of 5.5µm (n=10) and are surround by a variety of smaller microspheres between 1-2µm in diameter (n=10) (ratio: 1 Large : 2 smaller). The image also displays a large amount of small microspheres that have been formed surrounding the larger ones (Scale Bars : 2.00µm).

When analysing the micrographs in figure 5, it is evident that as the molecular weight of PCL decreases from 80kDa to 10kDa, the diameter of microspheres on average decreases. The PCL 80kDa microspheres show an approximate 8.4µm diameter, reducing to 5.5µm when PCL 10kDa was utilised. Visually after comparing the empty and FLT encapsulated microspheres to each other, regarding size there is a small reduction in particle size after FLT was incorporated.

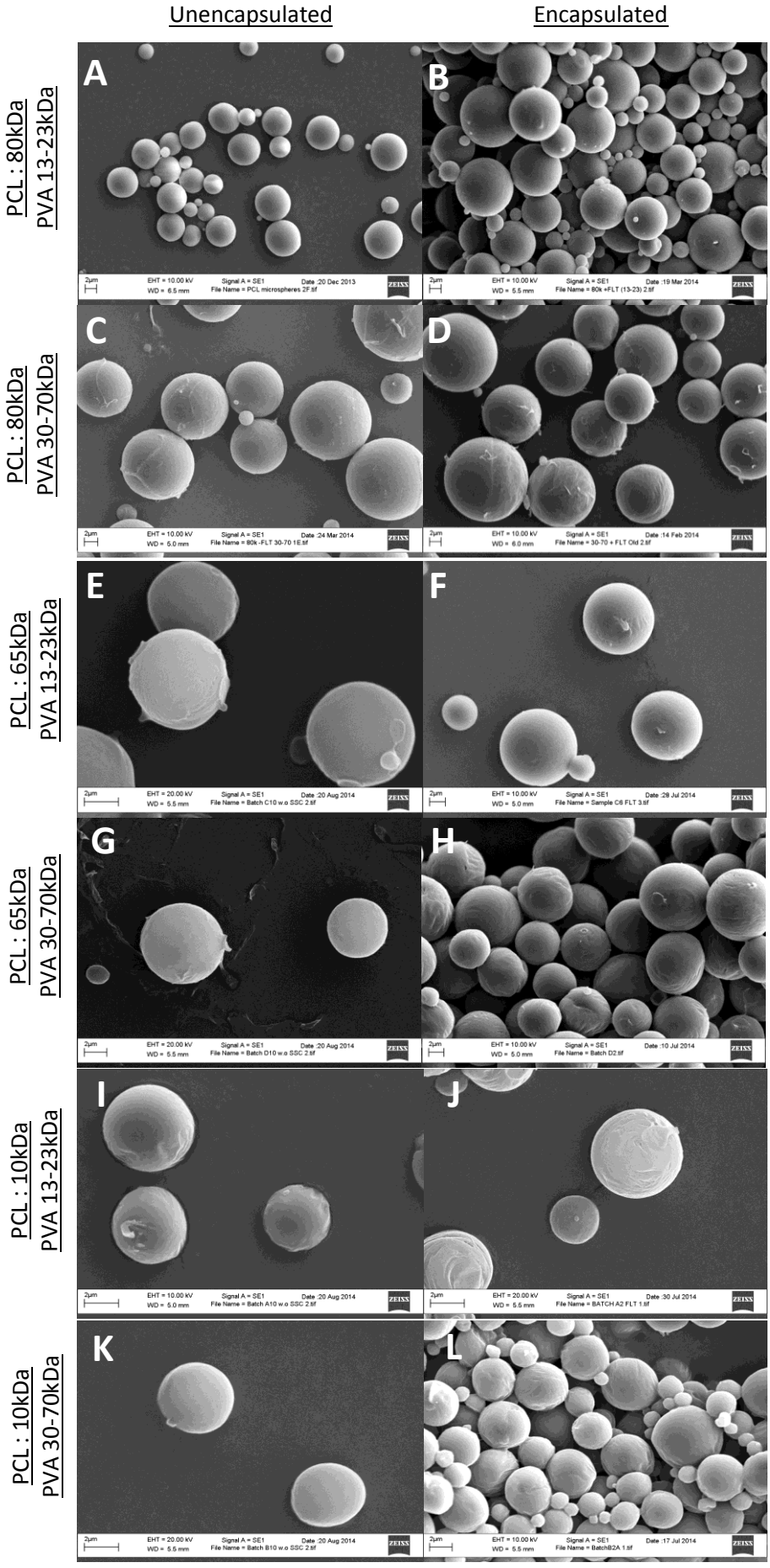


Figure 5. SEM Micrographs of Unencapsulated and Encapsulated Flutamide Microspheres. **A**= (4000x mag), **B**= (5000x mag), **C**= (5000x mag), **D**= (5000x mag), **E**= (12,000x mag), **F**= (10,000x mag), **G**= (12,000x mag), **H**= (10,000x mag), **I**= (12,000x mag), **J**= (12,000x mag), **K**= (12,000x mag), **L**= (10,000x mag). (See Appendix 3.1.1. for detailed formulations).

The PCL 80kDa formulation shown in figure 5 incorporated 2 molecular weights of PVA, both produced smooth microspheres. Figure 5 micrographs C and D incorporated a higher surfactant molecular weight range and displayed the most uniform microspheres amongst all the formulations. This visual analysis suggests that with a higher PVA molecular weight range (30-70kDa) increased uniformity of microspheres can be achieved. The effect of using PVA at a higher molecular weight range can be seen when comparing micrographs E, F to G, H and micrographs I, J to K, L.

Overall figure 5 micrographs C and D displayed the most ideal microspheres for dissolution analysis. After evaluating their morphology it suggests the PVA 30-70kDa at 0.5% wt/v has stabilised the aqueous phase during formulation, creating very uniform and spherical microspheres as seen from the SEM micrograph.

In figure 6 image **A** shows partially formed microspheres that are quite smooth but showing a large amount of clumping. Using the same experimental techniques throughout the formulation process, in this instance either not all the solvent has been evaporated or the drug did not fully encapsulate (Scale Bar : 2µm).

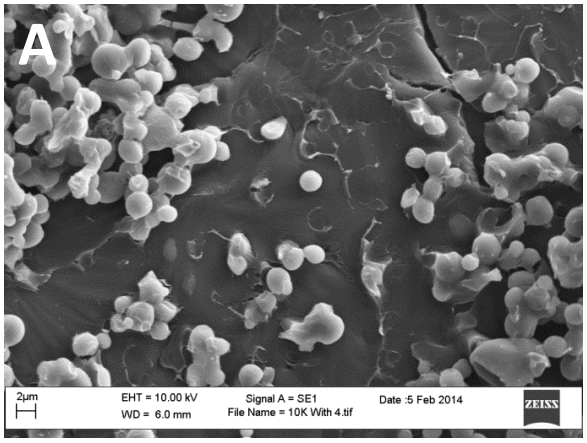


Figure 6. **A =** SEM image of the FLT loaded PCL 10kDa - PVA 30-70kDa microspheres (4,000x mag) (See Appendix 3.1.1. for detailed formulations).

3.2. Percentage Encapsulation Efficiency (Direct Method)

Table 2 shows 3 different PCL polymer molecular weights (80kDa, 65kDa and 10kDa), which are then subdivided into 2 PVA surfactant molecular weight ranges (13-23kDa and 30-70kDa). Each individual formulation of FLT loaded microspheres was then tested using the direct encapsulation method. This analysis was carried out in triplicate to calculate the average percentage (Appendix 3.2.1.) The two-tailed t-test p-value compared the percentage encapsulation efficiency of the PVA 13-23kDa formulations vs PVA 13-23kDa formulations. The definition of a statistical significance is a p-value that is <0.05 with a confidence interval of 95% or higher. A multiple comparison of column data was carried out using a Holm-Šídák's test within a one-way ANOVA analysis (Using Graph Pad Prism 6) to establish whether there is a significant difference between the control and other sets of data (Table 2). The Holm-Sidak's multiple comparison test confirmed via p-values that there is a significant difference between the control and the other the sets of data.

Table 2. A table displaying the direct percentage encapsulation efficiency of FLT in the test formulations (N=3) (Appendix 3.2.1.).

Direct Percentage Encapsulation Efficiency of FLT						
(Polymer)PCL MW	80kDa		65kDa		10kDa	
(Surfactant) PVA MW	13-23kDa	30-70kDa	13-23kDa	30-70kDa	13-23kDa	30-70kDa
Average (%)	**90.12 ±0.56**	**90.92 ±1.08**	**80.88 ±2.04**	**79.25 ±1.92**	**74.33 ±4.51**	**72.05 ±1.81**
(T-test) P value	0.3210		0.3701		0.4605	
Holm-Sidak's test (1)	Control		**		**	
Holm-Sidak's test (2)		Control		***		****

(ns : P>0.05, * : P≤0.05, ** : P≤0.01, *** : P≤0.001 or **** : P≤0.0001)

Figure 7 was created using the collected data displayed in table 2. Figure 7 proficiently illustrates that the over the three PCL molecular weights tested, 80kDa displayed the highest average %EE at 90.12% ±0.56% and 90.92% ±1.08%. As the molecular weight of PCL reduced the encapsulation efficiency percentage also reduced.

When analysing the effect of the two PVA surfactant molecular weights, PCL 80kDa/ PVA 30-70kDa encapsulated 0.80% more FLT in comparison to PVA 13-23kDa. PCL 65kDa/ 13-23kDa encapsulated 1.63% more FLT in comparison to PVA 30-70kDa. PCL 10kDa/ 13-23kDa encapsulated 2.25% more FLT in comparison to PVA 30-70kDa. The standard deviation was also calculated and plotted as standard error bars in figure 7. It suggests from the standard error that as the molecular weight of PCL reduced from 80kDa to 10kDa, the variance of percentage encapsulation efficiency in relation to the average increased.

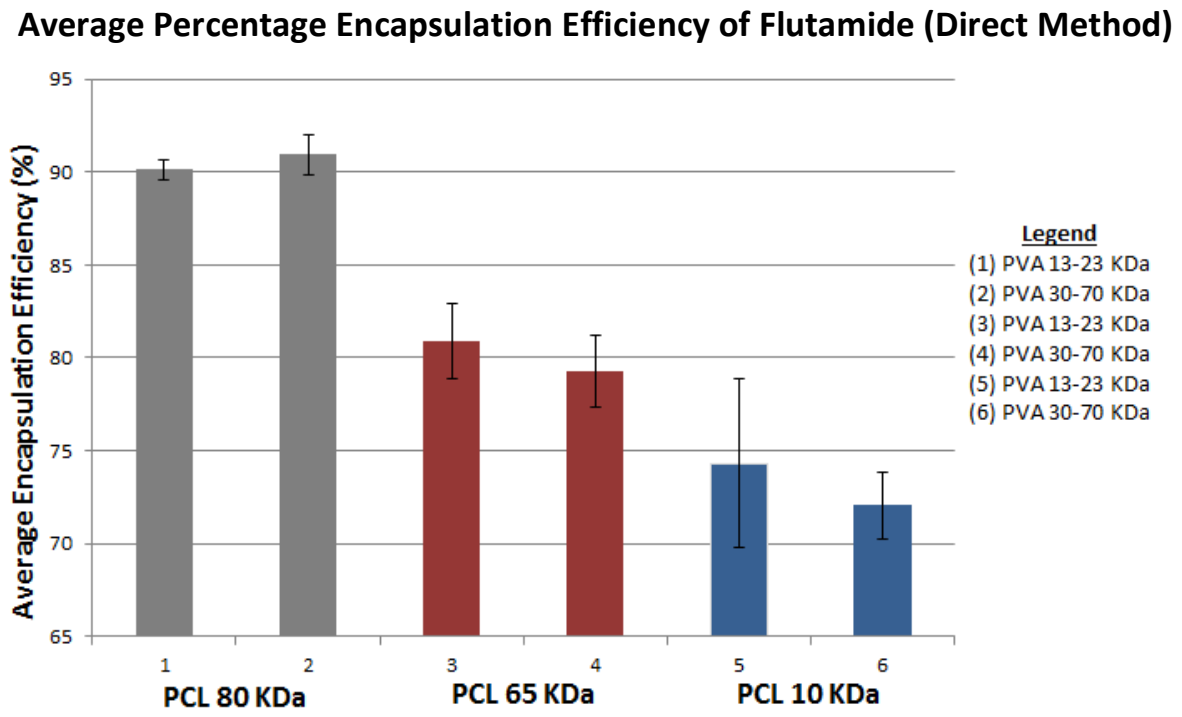


Figure 7. A graph showing the percentage encapsulation efficiency of FLT (using the direct method) in PCL microspheres (molecular weights : 80, 65 and 10kDa), each subdivided into two PVA molecular weights (molecular weights: 12-23 and 30-70kDa) (Appendix 3.2.1.).

3.3. Percentage Encapsulation Efficiency (Indirect Method)

This analysis was carried out in triplicate to see how much free FLT was left encapsulated. Using these readings the theoretical percentage of encapsulated FLT was indirectly calculated.

The indirect percentage encapsulation efficiency of FLT was calculated using the data shown in appendix 3.3.1. It was indirectly calculated that PCL 80kDa/ PVA 13-23kDa formulation gave an average percentage encapsulation of 92.20% ±0.22%, PCL 80kDa/ PVA 30-70kDa formulation gave an average percentage encapsulation of 90.52% ±1.44% (figure 8). The PCL 80kDa/ PVA 13-23kDa microspheres indirectly encapsulated 1.68% more FLT in comparison to PVA 30-70kDa. The standard deviation was also calculated and plotted as standard error bars in figure 8. It suggests from the standard error that formulation PVA 30-70kDa had a larger variance of %EE in relation to the average compared to the PVA 13-23kDa formulation.

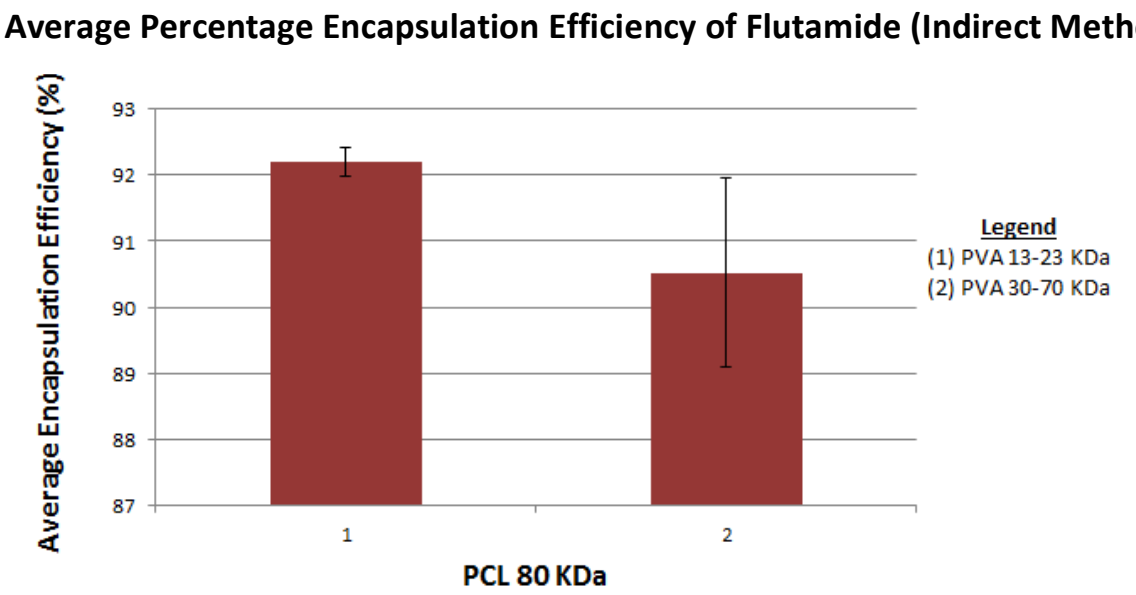


Figure 8. A graph showing the percentage encapsulation efficiency of FLT (using the indirect method) in PCL microspheres (molecular weight: 80kDa), which are subdivided into two PVA molecular weights ranges (molecular weights: 13-23 and 30-70kDa).

3.4. Percentage Yield

Table 3 displays the percentage yield of both empty and FLT loaded microspheres. Each formulation has 3 different PCL polymer molecular weights (80, 65 and 10kDa), which are then subdivided into 2 PVA surfactant molecular weight ranges (13-23 and 30-70kDa). When analysing the empty microsphere against the FLT loaded microsphere percentage yield, the percentage yield increases as drug is incorporated into the formulation.

In table 3 the average percentage yield for formulations that produced “empty microspheres” was found to be between 64% - 71%. The highest percentage yield of microspheres were formulated using PCL 80kDa and PVA 13-23kDa, achieving 70.46% **±**0.60% product. The lowest percentage yield of microspheres were formulated using PCL 10kDa and PVA 30-70kDa, displaying 63.92% ±2.02% product.

The average percentage yield for formulations that produced “FLT Loaded Microspheres” was between 64% - 73%. The highest percentage yield of microspheres were formulated using PCL 80kDa and PVA 13-23kDa, with a yield of 72.95% ±1.28%. The lowest percentage yield were microspheres that were formulated using PCL 10kDa and PVA 30-70kDa, displaying 64.81% ±0.87% product.

Table 3. A table displaying the percentage yield of the test formulations (N=3) (Appendix 3.4.1.).

Percentage Yield of Product						
Empty Microspheres						
(Polymer)PCL MW	80kDa		65kDa		10kDa	
(Surfactant) PVA MW	13-23kDa	30-70kDa	13-23kDa	30-70kDa	13-23kDa	30-70kDa
Average (%)	**70.46 ±0.60**	**70.11 ±1.61**	**69.71 ±4.37**	**69.39 ±1.05**	**64.94 ±1.42**	**63.92 ±2.02**
T-test (P value)	0.7258		0.9078		0.5128	
Holm-Sidak's test (1)	Control		ns		ns	
Holm-Sidak's test (2)		Control		ns		**
FLT Loaded Microspheres						
(Polymer)PCL MW	80kDa		65kDa		10kDa	
(Surfactant) PVA MW	13-23kDa	30-70kDa	13-23kDa	30-70kDa	13-23kDa	30-70kDa
Average (%)	**72.95 ±1.28**	**71.98 ±0.04**	**70.62 ±0.34**	**69.64 ±4.33**	**65.60 ±1.09**	**64.81 ±0.87**
T-test (P value)	0.2585		0.6790		0.3846	
Holm-Sidak's test (3)	Control		*		***	
Holm-Sidak's test (4)		Control		ns		*
Δ Average Percentage Yield (Average FLT Loaded Microspheres % - Average Empty Microspheres %)						
(Polymer)PCL MW	80kDa		65kDa		10kDa	
(Surfactant) PVA MW	13-23kDa	30-70kDa	13-23kDa	30-70kDa	13-23kDa	30-70kDa
Δ Average Percentage Yield **(%)**	**+2.49**	**+1.87**	**+0.91**	**+0.25**	**+0.66**	**+0.82**

(ns : P>0.05, * : P≤0.05, ** : P≤0.01, *** : P≤0.001 or **** : P≤0.0001)

3.5. Particle Size

The 3 different PCL polymer molecular weights (80, 65 and 10kDa), which are then subdivided into 2 PVA surfactant molecular weight ranges (13-23 and 30-70kDa) were assessed using a particle mastersizer (Malvern Mastersizer 3000 laser diffraction particle size analyser). The particle mastersizer is used to determine the size of microspheres that are present in the sample for both empty and drug loaded formulations. Figure 9 is a collaborative line graph of particle size that shows all the formulations, the highest peak in the graph displays what the average size of 90% of the sample was. It can be seen that as the PCL MW increases the peak shifts slightly to the right. This indicates that there is an increase in particle size as the PCL MW increases from 10kDa to 80kDa. The graph also indicates bimodal distribution, the highest peaks at 5.00-10.00µm (volume density 7-13%) and the shallow peaks at 0.01-0.20µm (volume density 1.00-3.00%). This shows that throughout all the formulations there were two groups of representable data. A small percentage of smaller micrsopheres and large percenatge of larger microspheres. The reason for the small percentage of smaller microspheres could be due to an excess amount PCL during the forulation stages. During the homogenisation process, excess PCL formed smaller PCL microspheres in emulsion. Another factor could be the homogenisation speed (5000rpm) being too high dispersing the emulsion more finely, creating smaller micropsheres.

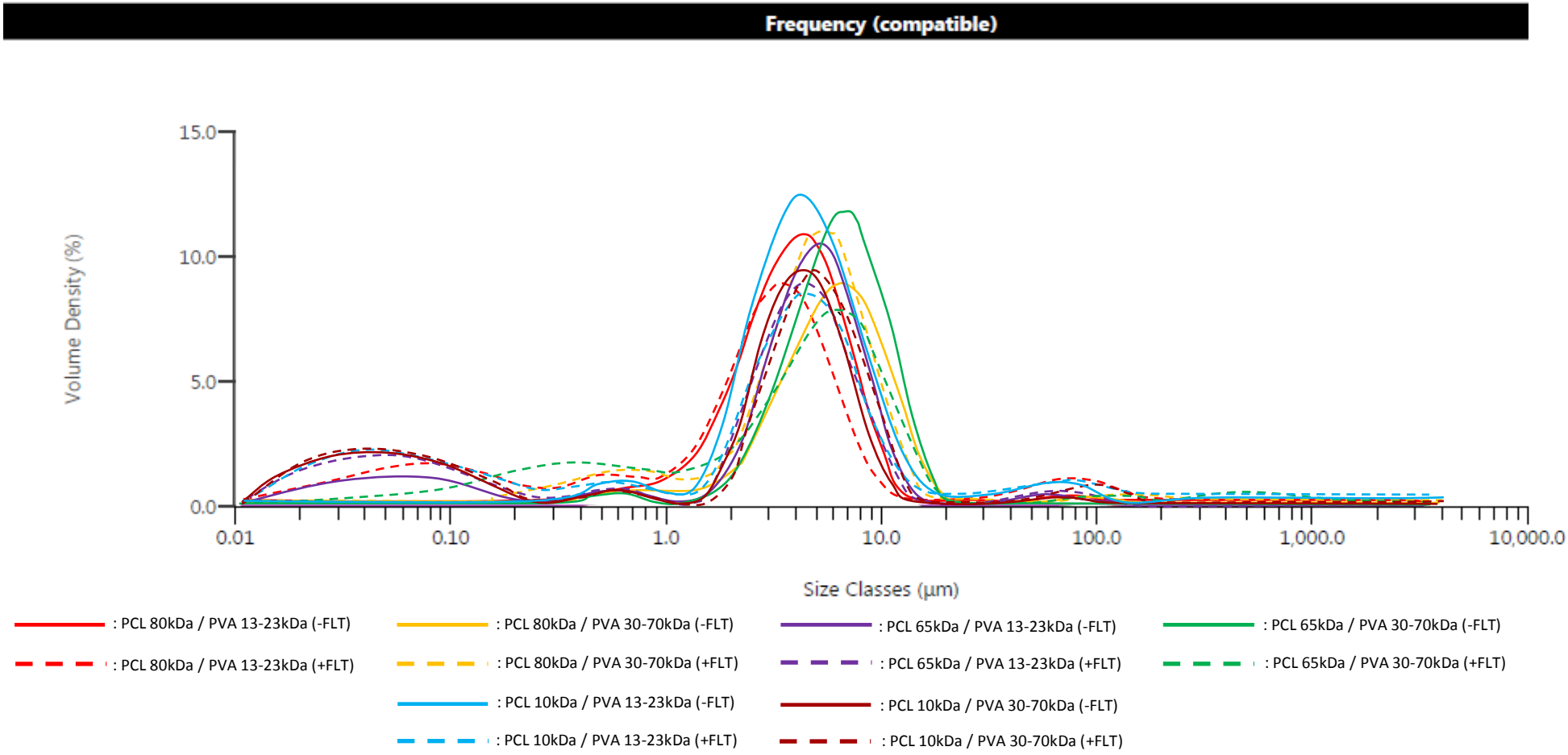


Figure 9. A collaborative line graph displaying the mastersizer data for all the microsphere formulations.

Using the Malvern Mastersizer 3000 it provided quantitative data regarding the size of microspheres. All samples displayed comparable microparticles ranging from 6.87µm to 11.50 µm (Dv90). When comparing the encapsulated drug formulations with the empty formulations, overall the morjority of formulations displayed a decrease in size when the drug was encaspsulated (Table 4).

Analysing 90% of each sample **(Dv90),** is displays that all the formulations shows a decrease in particle when comparing the "Empty" and "FLT Loaded" microspheres, excluding the PCL 65kDa/ PVA 30-70kDa and PCL 10kDa/ PVA 30-70kDa.

Table 4. A table displaying the particle sizes of the test formulations (N=5).

Particle Size (µm)						
Empty Microspheres						
(Polymer)PCL MW	80kDa		65kDa		10kDa	
(Surfactant) PVA MW	13-23kDa	30-70kDa	13-23kDa	30-70kDa	13-23kDa	30-70kDa
(Dv90) µm	8.23	10.40	8.63	10.90	8.39	6.87
FLT Loaded Microspheres						
(Polymer)PCL MW	80kDa		65kDa		10kDa	
(Surfactant) PVA MW	13-23kDa	30-70kDa	13-23kDa	30-70kDa	13-23kDa	30-70kDa
(Dv90) µm	7.35	8.25	7.90	11.50	8.20	7.45
Δ Particle Size (FLT Loaded Microspheres - Empty Microspheres)						
(Polymer)PCL MW	80kDa		65kDa		10kDa	
(Surfactant) PVA MW	13-23kDa	30-70kDa	13-23kDa	30-70kDa	13-23kDa	30-70kDa
(Dv90) µm	**-0.88**	**-2.15**	**-0.73**	**+0.60**	**-0.19**	**+0.58**

3.6. *In-Vitro* Release Studies

The active substance FLT was analysed using a spectrophotometer to produce a calibration curve as seen in figure 10. The curve is an average of triplet readings for each dilution to improve reliability of data. Initially when analysing the stock solution (FLT : 0.05 mg/mL ethanol) the spectrophotometer displayed a digital absorbance spectrum that peaked at exactly 300nm wavelength. The calibration graph in figure 10 produced a line equation (y=37.879x) that can be utilised to directly calculate the percentage FLT release at regular time intervals.

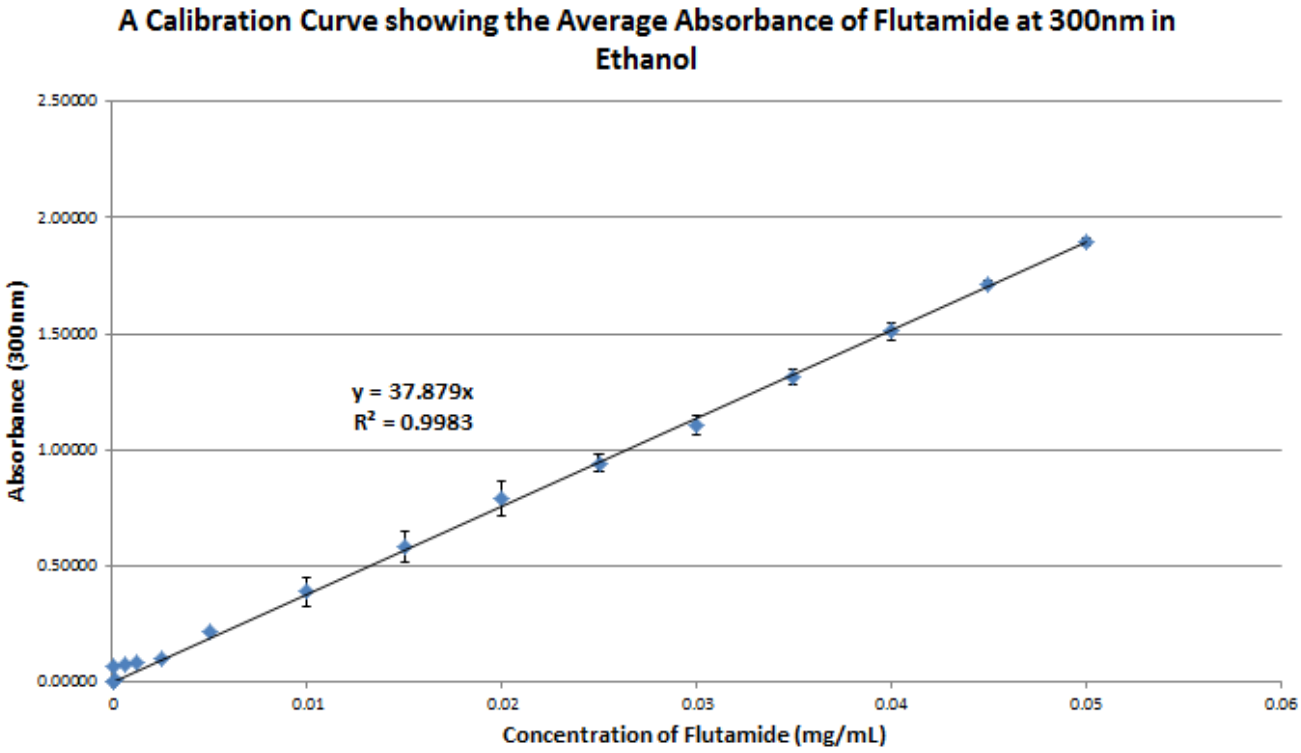


Figure 10. Regression analysis of the calibration curve for Flutamide showed a linear relationship between the intensity of fluorescence and the concentration using a spectrophotometer at 300nm (N=3) (Appendix 3.6.1.).

The *in-vitro* release profile of the microsphere formulations are encapsulated using different PCL molecular weights (80kDa, 65kDa and 10kDa). These different formations of microspheres are then subdivided into two different PVA molecular weights ranges, (13-23kDa and 30-70kDa).

This sigmoidal release profiles all have a common denominator, they display "first" order kinetics, suggesting, the drug release rate depends on its concentration. The release profiles in figure 11 express 6 sigmoidal curves with 6 maximal parentage releases (Appendix 3.6.2.; 3.6.3. & 3.6.4.) :

- PCL 80kDa-PVA 13-23kDa maximal release = 72.14% FLT.
- PCL 80kDa-PVA 30-70kDa maximal release = 80.23% FLT.

These maximal release readings were recorded after 16 days of dissolution until no more FLT was seen to be released. The supporting numerical data (Appendix 3.6.2.; 3.6.3. & 3.6.4) displays that the PCL 80kDa-PVA 13-23kDa formulation released about 19% of its active ingredient FLT within 4 hours of suspension. When analysing the PCL 80kDa-PVA 30-70kDa formulation data, it showed that it released about 15% of its encapsulated FLT within 4 hours of suspension.

- PCL 65kDa-PVA 13-23kDa maximal release = 59.14% FLT release after 9 days.
- PCL 65kDa-PVA 30-70kDa maximal release = 63.42% FLT release after 14 days.

The supporting numerical data (Appendix 3.6.2.; 3.6.3. & 3.6.4) also suggested that the PCL 65kDa-PVA 13-23kDa formulation released about 21% of its active ingredient FLT within 4 hours of

suspension. When analysing the PCL 65kDa-PVA 30-70kDa formulation data, it showed that it released about 12% of its encapsulated FLT within 4 hours of suspension.

- PCL 10kDa-PVA 13-23kDa maximal release = 31.24% FLT release after 4 days.
- PCL 10kDa-PVA 30-70kDa maximal release = 32.82% FLT release after 7 days.

The supporting numerical data (Appendix 3.6.2.; 3.6.3. & 3.6.4) also suggested that the PCL 10kDa-PVA 13-23kDa formulation released about 16% of its active ingredient FLT within 4 hours of suspension. When analysing the PCL 10kDa-PVA 30-70kDa formulation data, it showed that it released about 13% of its encapsulated FLT within 4 hours of suspension.

After analysing the data in appendix 3.6.2.; 3.6.3. & 2.6.4 and figure 11 the results show that as the molecular weight of PCL increases, the rate of release slows down. This can be seen as the PCL 80kDa formulation takes approximately 16 days to achieve maximal release. The PCL 10kDa released at a much faster rate with in only 4 days in comparison. In regards to the PVA molecular weight ranges it is significant that the PVA 30-70 molecular weight range causes an increase in maximal release throughout all the polymer molecular weights. It can also be determined from the release profiles in figure 11 that as the PCL molecular weight increases so does the maximal release percentage.

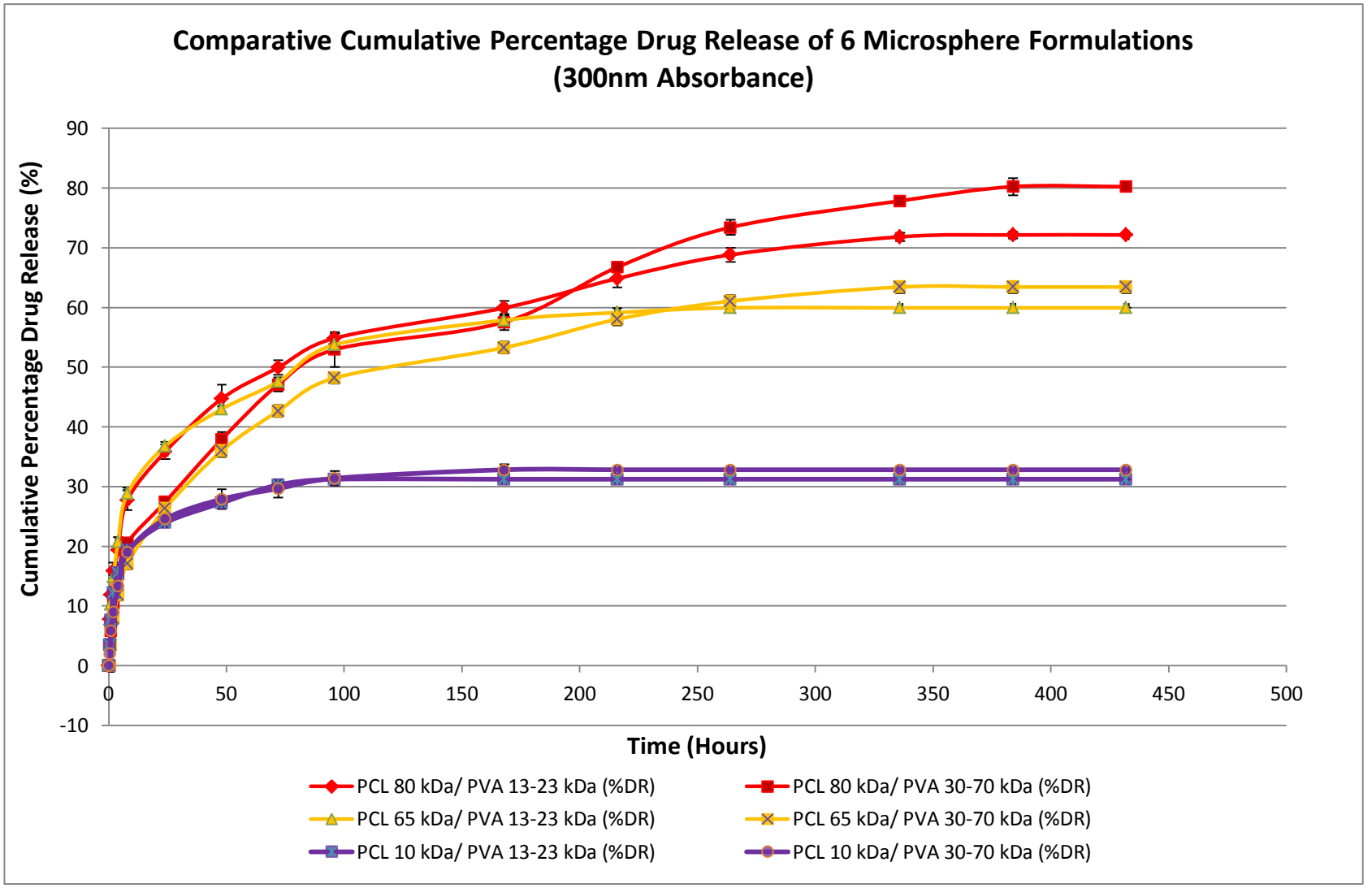


Figure 11. The *in-vitro* release profiles of all 6 FLT encapsulated microsphere samples.

4.0. Discussion

4.1. The Effects of PCL Molecular Weight on Particle Size.

Analysing the data gathered from the scanning electron microscope micrographs shown in figure 5 illustrate definitive features regarding aesthetics and size in relation to the different formulations. Figure 5 (A-L) demonstrate that as the PCL (polymer) decreased in molecular weight, visually there was also a small reduction in the microspheres diameter from 8.4µm to 5.5µm. The molecular weight of the polymer PCL is directly related to the chain length, as molecular weight decreases the chain length becomes shorter. The mechanical properties of the polymer are influenced by its molecular weight. To incorporate a polymer into the formulation that is strong and durable, the polymer has to have a molecular weight much larger than 10kDa for structural applications. When formulations are relating to thin films or other special applications such as microparticles, low molecular weight polymer or oligomer occasionally is adequate (Sui, 2013).

Sui's research in 2013 stated that a polymer must be considerably larger than 10kDa to be used as polymeric microparticles. During this investigation, it was noted that formulations that incorporated PCL at 10kDa molecular weight (figure 5, (I-L)) displayed a lack of strength when being analysed by the SEM. The lack of stability can be seen as the electron beam was focused over a region of microspheres, within 15-30 seconds the microspheres began to disintegrate under the electron beam. This indicated that due to the low molecular weight of PCL 10kDa the microspheres do not hold a significant amount of strength and stability compared to the other formulations. These properties mean that PCL 10kDa has a very thin matrix, affecting its stability during the SEM analysis. This indicated that PCL 10kDa microspheres will degrade in a biological environment at a much faster rate compared to higher PCL molecular weights formulations, which is not ideal for controlled release.

4.2. Drug Overloading

Figure 6, is an SEM image of microspheres using PCL 10kDa encapsulated with 50mg FLT. The image suggested that full encapsulation of FLT was unsuccessful. This has caused a large amount of clumping with unencapsulated free FLT. The non-spherical flakes and connective strands inbetween the irregular clumps of PCL display the unencapsulted FLT. Small amounts of drug can be seen bound to the carbon backing as a thin film. The formation of small and fragile microspheres has led to them being unable to capacitate 50µg FLT in comparison to other formulations that incorporate higher PCL molecular weights. It was stated by Wise during his research in 2000 when studying the controlled release of poly(lactic-co-glycolic acid) (PLGA) a similar polymer to PCL that, "low polymer concentrations and low molecular weight polymers lead to lower viscosity mixtures and favour the formation of smaller microspheres" (Wise, 2000). Other supporting studies are by Ravi. S and his team in 2008 and Tiwari. S and his team in 2011 who discovered that while producing PLGA microspheres, as the polymer molecular weight was increased so did the microsphere size (Ravi *et al*., 2008; Tiwari and Verma, 2011).

As a result of the data gained from the SEM micrograph in figure 6, a change in drug quantity was made when using PCL 10kDa. The drug quantity was amended from 50mg to 25mg, thus showing a significant improvement regarding microsphere formation and the amount of free drug left unencapsulted. A review paper by Metkar. V.B. and his team in 2014 states that "It was found that highest drug loading in microspheres may be achieved by incorporating the drug through the time of preparation but it may get affected by many other process variables like presence of additives, method of preparation, heat of polymerization, agitation intensity etc" (Metkar *et al*., 2014). In regards to the evidence found, by reducing the affecting variable (quantity of FLT during preparation) improved the microsphere yield using PCL 10kDa. Over loading microspheres of small size with active ingredients can reduce the efficiency of drug encapsulation, hence why a reduced amount of FLT (25 µg) was used for this particular formulation.

4.3. The Effect of Using PVA 0.5% wt/v

Looking at the effect of the two different molecular weight ranges of PVA (13-23 and 30-70kDa) throughout the test formulations visually shows a significant change in morphology. In comparison to other studies by Ozlem Aydin in 2014, it was concluded that "spherical forms could be obtained with 1, 2, 4, and 6% wt/v PVA, but not with 0.5%" (Aydin *et al*., 2014). This investigation incorporated PVA 0.5% wt/v and spherical microspheres can be seen from the SEM micrographs in figure 5, therefore suggesting an alternative result.

The SEM micrographs in figure 5 display that the surfactant has played a vital role in the stabilising of the primary emulsion. The result of incorporating PVA can be seen in Figure 5 - micrograph C-D, the microspheres are smooth and are very uniform in regards to size and distribution.

4.4. Comparative Percentage Encapsulation Efficiency (Direct Method).

The direct percentage encapsulation efficiency (%EE) of all the formulations shown in table 2 was found to be between 72.00% - 91.00%. When analysing the average %EE of formulations that incorporated the low molecular weight of PCL 10kDa, it was established that the microspheres could encapsulate 72.05% +/- 1.81% (PVA : 30-70kDa) and 74.33% +/- 4.51% (PVA : 13-23kDa) of FLT. In comparison to a study that investigated a drug delivery system of doxycycline-loaded polycaprolactone (PCL) microspheres in 2014 by Ozlem Aydin. The encapsulation of doxycycline using PCL 14kDa polymer produced microspheres that achieved an average %EE of 52.79% (Aydin *et al*., 2014). Although the PCL polymer molecular weights were not identical but still very similar (PCL 10kDa against 14kDa), the data found from this investigation in comparison to Ozlem Aydin's research shows ≈20% more drug can successfully be encapsulated into microspheres made with PCL 10kDa. Although it can be seen that the PVA 13-23kDa formulations encapsulated more FLT into the microspheres compared to the PVA 30-70kDa formulations, after conducting a t-test a p-value of 0.4605 signifies there is no significant difference between the two sets of data.

Table 2 displays formulations that incorporated PCL 65kDa, it was established that the microspheres could encapsulate 79.25% +/- 1.92% (PVA : 30-70kDa) and 80.88% +/- 2.04% (PVA : 13-23kDa) of FLT. Ozlem Aydin investigated the encapsulation of doxycycline using PCL 65kDa and it was established from the 2014 study that the produced microspheres had an %EE of 73.57% (Aydin *et al.*, 2014). The data found from this investigation successfully encapsulated ≈7% more drug in PCL 65kDa microspheres when compared to Ozlem Aydin's research. Although it can be seen that the PVA 13-23kDa sample encapsulated more FLT into the microspheres compared to the PVA 30-70kDa formulation, after conducting a t-test a p-value of 0.3701 signifies there is no significant difference between the two sets of data.

The final formulation that incorporated PCL 80kDa showed microspheres that could encapsulate 90.92% +/- 1.08% (PVA : 30-70kDa) and 90.12% +/- 0.56% (PVA : 13-23kDa) of FLT. In comparison Liaqat Ali's study from 2014 investigated the development of biodegradable PCL microspheres for controlled release of venlafaxine. It was established from the study that the produced PCL 80kDa microspheres achieved an %EE of 71.29% (Ali *et al.*, 2014). The data found from this investigation successfully encapsulated ≈20% more drug in PCL 80kDa microspheres when compared to Liaqat Ali's research. Although it can be seen that the PVA 30-70kDa sample encapsulated slightly more FLT into the microspheres compared to the PVA 13-23kDa formulation, after conducting a t-test a p-value of 0.3210 signifies there is no significant difference between the two sets of data.

Comparing the %EE from all the formulations proves that the larger PCL molecular weight formulations encapsulate more FLT in comparison to the 65kDa or 10kDa formulations. Statistically the difference in %EE in relation to the PCL molecular weight is very significant as $P \leq 0.01$..

A benefit of improved encapsulation efficiency of PCL-FLT microspheres is that more of the active agent reaches the blood plasma to cause effect at the target site (testicles). This also means less FLT is needed in each dose, being more economically beneficial for the supplier. The controlled release microsphere system gives the benefit of reducing toxic concentrations of FLT in the blood plasma, reducing the likelihood of harsh side effects.

4.5. The Effects of PCL Molecular Weight on Product Yield.

Both "Empty" and "FLT Loaded Microspheres" decrease in product yield as the PCL molecular weight decreases. The reasoning for this is due to the reduction in microsphere diameter as the PCL molecular weight was reduced in formulation (PCL 80kDa → 65kDa → 10kDa). Another factor is that the likelihood of aggregation decreases as the mean diameter of the microspheres increases, because Brownian motion of smaller microspheres makes hydrophobic interactions more likely (Bang Laboratories. 2013). This theory shows that aggregation increases as the diameter of microspheres decrease, therefore making it harder to yield product as clumping is more likely to occur. Other than the PCL molecular weight, another viable reason for decreased production yield is a using a higher drug:polymer ratio (PCL 10kDa = 1:20) which causes a decreased diffusion rate of the solvent (chloroform) from concentrated solutions into initial emulsion (Youan *et al*., 2001).

4.6. Factors Effecting Microsphere Size.

The properties that are found in the aqueous phase are not the only factors that control microsphere size for further optimization of microspheres. The stirring speed has been found in other studies to affect particle size as it gives energy to disperse the organic phase more in water. A layer of foam on top of the solution had been produced due to the high stirring rate, the solution was left for 5 minutes to settle to reduce any excessive foam. Microsphere sizes were small in diameter due to the high stirring rate (5000 rpm) during the homogenisation process, as it results in

the formation of finer emulsions by breaking it up into smaller droplets (Aishwarya *et al*., 2008; Wang *et al*., 2008). In studies when the concentration of PVA was changed from 0.25% to 2.0% wt/v, microsphere sizes also decreased (Vivek *et al*., 2007). Therefore throughout this investigation the percentage concentration of PVA remained constant at 0.5% wt/v, to ultimately produce microspheres within a strict diameter range.

Overall seen from table 4 the data displays that using a higher PVA molecular weight range (30-70kDa) a microsphere size increase can been achieved. A trend can be seen between the PCL MW and microsphere size, the higher PCL MW the larger the particle size. Jong-Keol Jeong and his team in 2003 when studying the release behaviour of PCL for drug release behaviour also discussed that using a higher PCL MW increases the particle size (Jeong *et al*., 2003).

4.7. Factors Effecting Initial Burst Release and Maximal Release.

The initial 4 hour dissolution period of formulation PCL 80kDa - PVA 13-23 (19 %DR)kDa and PCL 65kDa - PVA 13-23kDa (21 %DR) presented the fastest burst release. A factor related to porosity is the previously mentioned initial burst effect within the first 4 hours of dissolution. This corresponds to a rapid initial release of the drug and is normally followed by a relatively-controlled linear release. This initial burst of FLT is attributed to leaching which occurs at the outer wall of the sphere as it becomes hydrated by the surrounding medium (LeCorre *et al*., 1994; Okada *et al*., 1994 and Ghaderi *et al*., 1996).

According to the release profiles produced in this study, the largest initial burst of FLT in the first 4 hours burst theoretically should have been in the formulations that incorporated PCL 10kDa. This is due to the porosity of the microsphere as it has a thinner matrix allowing medium to diffuse through the shell hydrating it. However this did not occur, the reasoning for this anomaly may be due to the excess unencapsulated free FLT that can attach to the exterior surface of the microspheres. This

suggests that any surface bound FLT will induce a larger initial burst release, as shown for PCL 80kDa formulations. Supporting evidence from this study can be found in Figure 5, in micrographs A-H where is displays small surface flakes on the exterior surface of the microspheres which indicate un-encapsulated free FLT, suggesting that 100% encapsulation may not have been achieved.

Overall the formulations that incorporated PVA 30-70kDa proved to have a higher maximal controlled release but had a lower initial burst of FLT compared to PVA 13-23kDa formulations. Microspheres that were formed using PCL 80kDa displayed the highest deviation in max drug release percentage between the two PVA molecular weight ranges, the PCL 80kDa-PVA 30-70kDa formulation showed ≈8% increased percentage release. Previous studies have suggested that increasing "concentrations" of PVA decreased the initial burst of protein and the overall release rates (Jain *et al*., 2000). However external studies haven't investigated two different PVA molecular weight ranges and compared there effects regarding release studies as shown in this investigation (Appendix: 3.6.2., 3.6.3. and 3.6.4.).

4.8. The Effects of PCL Molecular Weight on Drug Release Rate.

In this study the rate of release was dependant on the PCL molecular weight used. It was seen that the higher the PCL molecular weight the slower the rate of release overall. As mentioned by Bezemer, J.M and his team of researches during their study into microspheres and protein delivery, "low-molecular-weight polymer resulted in porous, quickly releasing microspheres while the high-molecular-weight formulation resulted in dense microspheres and produced a sigmoidal release profile" (Bezemer *et al*., 2000).

The polymer molecular weight affects the polymers degradation and drug release rates. Review papers have stated that an increase in molecular weight decreases diffusivity and therefore drug release rate (LeCorre *et al*., 1994; Liggins and Burt, 2001). A mechanism for release of the active drug

is diffusion through water filled pores, formed as polymer degradation generates soluble monomers and oligomers that can diffuse out of the particle. These smaller products are produced more readily during the degradation of lower molecular weight polymers such as PCL 10kDa (Blanco and Alonso, 1998).

Another comparison can be made to this study by Jong-Keol Jeong and his team who also indicated that another factor mainly acted on drug release besides a particle size effect. The release behaviour seen is because the PCL molecular weight was high, the amorphous region will be wide open and form a coarse crystalline microstructure through which the drug will diffuse rapidly. Therefore it was determined by Jong and his team that the internal crystalline microstructure compared with particle size effect plays an important role in drug release (Jeong *et al*., 2003).

5.0. Conclusions and Further Studies

5.1. Conclusions

This study concludes that we successfully prepared, developed and tested a controlled drug delivery systems consisting of Poly-(*epsilon*-caprolactone) encapsulating the prostate cancer chemotherapy drug FLT. The development stage allowed the microspheres formulation to be refined, taking into account the poly-(*epsilon*-caprolactone) molecular weight and the poly vinyl alcohol molecular weight range.

Aesthetically from the SEM analysis it proves that a high PCL molecular weight (MW) (80kDa) at 0.5% wt/v PVA concentration and a high PVA MW range (30-70kDa) produce the most ideal drug delivery system. The microspheres are smooth, spherical in shape, uniform in size and well distributed.

Table 2 displays that when a lower PVA molceular weight range is used (13-23kDa) the percentage encapsultion of FLT is 1.60% higher on average compared to when PVA 30-70kDa is used. However after staistical analysis (t-test) the p-value suggests that there is no significant difference ($p>0.05$) between the PVA molecular weight ranges. The same sinerio can be seen in table 3 visually when PVA 13-23kDa, it yields 1.00% on average more product in comparison to PVA 30-70kDa. Again after staistical analysis (t-test) the p-value suggests that there is no significant difference ($P>0.05$) between the PVA molecular weight ranges. The SEM micrographs and mastersizer samples have shown a small increment in particle size when the 80kDa PCL was used.

Dissolution testing concluded that the higher PCL MW (80kDa) used the larger the maximal release, the slower the release rate of FLT and the higher the initial burst release. The initial burst release data has suggested that further research will be needed to solidify the outcome in this study, for example an alternative formulation method to reduce external surface drug (double emulsion encapsulation). In regards to the affects of PVA the dissolution data confirms that a higher PVA MW range increases the maximal release through out all the formulations. When encapsulating within a low PCL MW polymer (10kDa) over encapsulation can occur causing clumping and a high percentage of unencapsulated free FLT. To overcome this problem, drug content was reduced to form uniform, spherical microspheres for further testing. The use of 0.5% wt/v of PVA has proven sucessful in this study and can be used to contradict other studies that have previously confirmed that 0.5% wt/v of PVA is insuficient to create spherical microspheres.

A definitive single formulation can be selected from this study but further study is required. To conclude, the sample that has produced optimal data for a future controlled drug delivery system is the PCL 80kDa/ PVA 30-70kDa formulation. This formulation can be administered less frequently at a much lower dose (50mg), as the degredation rate (16 days) of PCL 80kDa is much slower than the

FLT half life (8 hours). After further studies are carried out the synthesized delivery system may offer a successful and promising potential application for many diseases such as prostate cancer.

5.2. Further Studies

The conclusions have defined the success of this study; improvements are still needed to solidify the validity of the data collected. Looking at only the PCL 80kDa microspheres, further study will provide even more evidence to back up the integrity of the product.

The first analysis to further the investigation will be transmission electron microscopy (TEM) analysis. This will show a cross sectional view of an individual microsphere and how FLT has been distributed throughout the microspheres core and also show the thickness of the matrix. Zhang and his team of researchers in 2003 successfully carried out TEM analysis on PLGA: PLLA polymer microspheres. Their TEM micrographs show a cross section of the microspheres allowing you see the enacapsulated Etanidazole (Zhang *et al*., 2003).

Another development is looking into altering the formulation by encapsulating the active ingredient (Flutamide) within a co-polymer/diblock or triblock-polymer rather than a single PCL polymer as other studies show promising data. Cao and Shoichet while studying the "Delivering neuroactive molecules from biodegradable microspheres for application in central nervous system disorders", displayed successful formulations when using a PCL-PLGA (50:50) diblock co-polymer (Cao and Shoichet, 1999).

A final development can be using *in-vitro* cytotoxicity testing such as MTT assay, this is a colorimetric test that analyses the drug delivery systems toxicity over a time period using cancer cell lines. (MTT assay protocol: Appendix 5.2.1.). This will indicate what dosage/dilution of PCL-FLT microspheres is therapeutic a specific time frame, causing cell apoptosis via the transcription factor

KLF9 which inhibits AKT activation and supresses tumor growth in prostate cancer patients (Shen *et al*., 2014).

6.0. References

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Notes

- Authentic flutamide can be obtained from Sigma-Aldrich, product no. F-9397.

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7.0. Appendices

Appendix : 3.1.1.

Figure 5: Image (**A**) Formulation :

- **Batch :** 2
- **Drug :** No drug
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **80kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **13-23kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**B**) Formulation :

- **Batch :** 1
- **Drug :** Flutamide - (50 µg)
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **80kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **13-23kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Drug : Polymer Ratio =** 1 : 10
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**C**) Formulation :

- **Batch :** 4
- **Drug :** No drug
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **80kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **30-70kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**D**) Formulation :

- **Batch :** 3
- **Drug :** Flutamide - (50 µg)
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **80kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **30-70kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Drug : Polymer Ratio =** 1 : 10
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**E**) Formulation :

- **Batch :** C10
- **Drug :** No drug
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **65kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **13-23kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**F**) Formulation :

- **Batch :** C6
- **Drug :** Flutamide - (50 µg)
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **65kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **13-23kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Drug : Polymer Ratio =** 1 : 10
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**G**) Formulation :

- **Batch :** D10
- **Drug :** No drug
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **65kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **30-70kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**H**) Formulation :

- **Batch :** D
- **Drug :** Flutamide - (50 mg)
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **65kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **30-70kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Drug : Polymer Ratio =** 1 : 10
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**I**) Formulation :

- **Batch :** A10
- **Drug :** No Drug
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **10kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **13-23kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Drug : Polymer Ratio =** 1 : 10
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**J**) Formulation :

- **Batch :** A2
- **Drug :** Flutamide - (25 mg)
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **10kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **13-23kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**K**) Formulation :

- **Batch :** B10
- **Drug :** No Drug
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **10kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **30-70kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Drug : Polymer Ratio =** 1 : 20
- **Oil : Water Ratio =** 1 : 30

Figure 5: Image (**L**) Formulation :

- **Batch :** B2A
- **Drug :** Flutamide - (25 mg)
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **10kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **30-70kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Oil : Water Ratio =** 1 : 30

Figure 6: Image (**A**) Formulation :

- **Batch :** B22
- **Drug :** Flutamide - (50 mg)
- **Solvent :** Chloroform - (10.0 mL)
- **Polymer :** *Poly*-(epsilon-*caprolactone*) **10kDa** - (500 mg)
- **Surfactant :** Poly Vinyl Alcohol **30-70kDa** - (1250 mg)
- **Homogenisation Speed**: 5,000 rpm
- **Drug : Polymer Ratio =** 1 : 20
- **Oil : Water Ratio =** 1 : 3

Appendix : 3.2.1.

Direct Percentage Encapsulation Efficiency Calculation Tables

PCL 80kDa / PVA 13 - 23kDa					
Batch 1	Sample A	Sample B	Sample C		Standard Curve of FLT:
Abs 300nm	0.754	0.795	0.711		
[FLT] cuvette	0.019905489	0.020987882	0.018770295		
Initial mg FLT	0.497637213	0.524697062	0.469257372		
Real mg FLT/mg FLT	4.602362787	4.475302938	4.530742628		y = 37.879x R^2 = 0.9983
Theoretical mg FLT/mg FLT	5.1	5.0	5.0	Average	
%EE	**90.24240758**	**89.50605877**	**90.61485256**	**90.1211063**	

PCL 80kDa / PVA 30 - 70kDa					
Batch 3	Sample A	Sample B	Sample C		Standard Curve of FLT:
Abs 300nm	0.751	0.615	0.667		
[FLT] cuvette	0.019826289	0.016235909	0.017608701		
Initial mg FLT	0.495657224	0.405897727	0.440217535		
Real mg FLT/mg FLT	4.404342776	4.694102273	4.359782465		y = 37.879x R^2 = 0.9983
Theoretical mg FLT/mg FLT	4.9	5.1	4.8	Average	
%EE	**89.88454644**	**92.04122104**	**90.82880136**	**90.91818961**	

PCL 65kDa / PVA 13 - 23kDa					
Batch C6	Sample A	Sample B	Sample C		Standard Curve of FLT:
Abs 300nm	1.305	1.456	1.548		
[FLT] cuvette	0.034451807	0.038438185	0.040866971		
Initial mg FLT	0.861295177	0.960954619	1.021674279		
Real mg FLT/mg FLT	4.138704823	4.139045381	3.778325721		y = 37.879x R^2 = 0.9983
Theoretical mg FLT/mg FLT	5.0	5.1	4.8	Average	
%EE	**82.77409647**	**81.15775258**	**78.7151192**	**80.88232275**	

PCL 65kDa / PVA 30 - 70kDa						
Batch D	Sample A	Sample B	Sample C			Standard Curve of FLT:
Abs 300nm	1.566	1.515	1.651			
[FLT] cuvette	0.041342168	0.039995776	0.043586156			
Initial mg FLT	1.033554212	0.999894401	1.089653898			
Real mg FLT/mg FLT	3.966445788	4.300105599	3.710346102			y = 37.879x R² = 0.9983
Theoretical mg FLT/mg FLT	5.0	5.3	4.8	Average		
%EE	**79.32891576**	**81.13406791**	**77.29887713**	**79.2539536**		

PCL 10kDa / PVA 13 - 23kDa						
Batch A2	Sample A	Sample B	Sample C			Standard Curve of FLT:
Abs 300nm	1.921	2.254	1.515			
[FLT] cuvette	0.050714116	0.059505267	0.039995776			
Initial mg FLT	1.2678529	1.487631669	0.999894401			
Real mg FLT/mg FLT	3.5321471	3.512368331	3.800105599			y = 37.879x R² = 0.9983
Theoretical mg FLT/mg FLT	4.8	5.0	4.8	Average		
%EE	**73.58639792**	**70.24736661**	**79.16886665**	**74.3342104**		

PCL 10kDa / PVA 30 - 70kDa						
Batch B2A	Sample A	Sample B	Sample C			Standard Curve of FLT:
Abs 300nm	2.258	2.151	1.985			
[FLT] cuvette	0.059610866	0.056786082	0.052403707			
Initial mg FLT	1.490271654	1.41965205	1.310092663			
Real mg FLT/mg FLT	3.509728346	3.68034795	3.689907337			y = 37.879x R² = 0.9983
Theoretical mg FLT/mg FLT	5.0	5.1	5.0	Average		
%EE	**70.19456691**	**72.1636853**	**73.79814673**	**72.05213298**		

Appendix : 3.3.1.

Indirect Percentage Encapsulation Efficiency Calculation Tables

PCL 80kDa / PVA 13 - 23kDa						
Batch 1	Sample A	Sample B	Sample C			Standard Curve of FLT:
Abs 300nm	0.363	0.383	0.38			y = 37.879x R^2 = 0.9983
[FLT] cuvette	0.009583146	0.010111143	0.010031944			
Initial mg FLT	3.833258534	4.044457351	4.012777528			
Real mg FLT/mg FLT	46.66674147	46.45554265	46.48722247			
Theoretical mg FLT/mg FLT	50.5	50.5	50.5	Average		
%EE	**92.40938904**	**91.99117356**	**92.05390588**	**92.2002813**		

PCL 80kDa / PVA 30 - 70kDa						
Batch 3	Sample A	Sample B	Sample C			Standard Curve of FLT:
Abs 300nm	0.442	0.460	0.333			y = 37.879x R^2 = 0.9983
[FLT] cuvette	0.011662885	0.012137844	0.008786743			
Initial mg FLT	4.665153834	4.855137474	3.514697346			
Real mg FLT/mg FLT	45.53484617	45.34486253	46.68530265			
Theoretical mg FLT/mg FLT	50.2	50.2	50.2	Average		
%EE	**90.70686487**	**90.32841141**	**92.99861087**	**90.51763814**		

Appendix : 3.4.1.

Percentage Yield Calculation Tables

80kDa With FLT (PVA 13 -23kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6738	14.7701
	2	14.7149	14.8103
	3	14.6917	14.7938
	4	14.7541	14.8625
Total		58.8345	59.2367
Dry Weight - Test Tube (g)		0.4022	
Mass of PCL in Preparation (g)		0.5060	
Mass of FLT in Preparation (g)		0.0553	
Total (PCL +/- FLT) (g)		0.5613	
Percentage Yield (%)		71.66	

80kDa With FLT (PVA 13 -23kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6800	14.8021
	2	14.6269	14.7591
	3	14.7694	14.8152
	4	14.7510	14.8599
Total		58.9158	59.3202
Dry Weight - Test Tube (g)		0.4044	
Mass of PCL in Preparation (g)		0.5033	
Mass of FLT in Preparation (g)		0.0508	
Total (PCL +/- FLT) (g)		0.5541	
Percentage Yield (%)		72.98	

80kDa With FLT (PVA 13 -23kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6800	14.8021
	2	14.6269	14.7591
	3	14.7694	14.8152
	4	14.7510	14.8599
Total		58.8273	59.2363
Dry Weight - Test Tube (g)		0.4090	
Mass of PCL in Preparation (g)		0.4999	
Mass of FLT in Preparation (g)		0.0512	
Total (PCL +/- FLT) (g)		0.5511	
Percentage Yield (%)		74.22	
Average Percentage Yield (%) (3 Sample)		**72.95**	

80kDa Without FLT (PVA 13 -23kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7159	14.8172
	2	14.7558	14.8267
	3	14.7268	14.8181
	4	14.7545	14.8460
Total		58.9530	59.3080
Dry Weight - Test Tube (g)		0.3550	
Mass of PCL in Preparation (g)		0.5006	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5006	
Percentage Yield (%)		70.91	

80kDa Without FLT (PVA 13 -23kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6789	14.7872
	2	14.6691	14.7131
	3	14.7852	14.8096
	4	14.8453	15.0190
Total		58.9785	59.3289
Dry Weight - Test Tube (g)		0.3504	
Mass of PCL in Preparation (g)		0.5022	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5022	
Percentage Yield (%)		69.77	

80kDa Without FLT (PVA 13 -23kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.4599	14.6587
	2	14.6641	14.6615
	3	14.6573	14.7124
	4	14.6854	14.7876
Total		58.4667	58.8202
Dry Weight - Test Tube (g)		0.3535	
Mass of PCL in Preparation (g)		0.5001	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5001	
Percentage Yield (%)		70.69	
Average Percentage Yield (%) (3 Sample)		**70.46**	

80kDa With FLT (PVA 30 -70kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7761	14.8834
	2	14.7521	14.8545
	3	14.8448	14.9442
	4	14.8021	14.8942
Total		59.1751	59.5763
Dry Weight - Test Tube (g)		0.4012	
Mass of PCL in Preparation (g)		0.5078	
Mass of FLT in Preparation (g)		0.0492	
Total (PCL +/- FLT) (g)		0.5570	
Percentage Yield (%)		72.03	

80kDa With FLT (PVA 30 -70kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7130	14.8021
	2	14.3201	14.4052
	3	14.3470	14.4689
	4	14.2594	14.3671
Total		57.6395	58.0433
Dry Weight - Test Tube (g)		0.4038	
Mass of PCL in Preparation (g)		0.5098	
Mass of FLT in Preparation (g)		0.0514	
Total (PCL +/- FLT) (g)		0.5612	
Percentage Yield (%)		71.95	

80kDa With FLT (PVA 30 - 70kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6525	14.7639
	2	14.6202	14.7376
	3	14.5776	14.6578
	4	14.7010	14.7944
Total		58.5513	58.9537
Dry Weight - Test Tube (g)		0.4024	
Mass of PCL in Preparation (g)		0.5090	
Mass of FLT in Preparation (g)		0.0502	
Total (PCL +/- FLT) (g)		0.5592	
Percentage Yield (%)		71.96	
Average Percentage Yield (%) (3 Sample)		**71.98**	

80kDa Without FLT (PVA 30 -70kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7053	14.7958
	2	14.7105	14.8115
	3	14.6977	14.7804
	4	14.7227	14.8109
Total		58.8362	59.1986
Dry Weight - Test Tube (g)		0.3624	
Mass of PCL in Preparation (g)		0.5059	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5059	
Percentage Yield (%)		71.63	

80kDa Without FLT (PVA 30 -70kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6385	14.6813
	2	14.6824	14.7942
	3	14.7076	14.7983
	4	14.7208	14.8280
Total		58.7493	59.1018
Dry Weight - Test Tube (g)		0.3525	
Mass of PCL in Preparation (g)		0.5152	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5152	
Percentage Yield (%)		68.42	

80kDa Without FLT (PVA 30 - 70kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6671	14.7766
	2	14.7189	14.8154
	3	14.7113	14.8022
	4	14.7217	14.7784
Total		58.8190	59.1726
Dry Weight - Test Tube (g)		0.3536	
Mass of PCL in Preparation (g)		0.5031	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5031	
Percentage Yield (%)		70.28	
Average Percentage Yield (%) (3 Sample)		70.11	

65kDa With FLT (PVA 13 -23kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7541	14.8650
	2	14.6941	14.7805
	3	14.7267	14.8247
	4	14.7340	14.8290
Total		58.9089	59.2992
Dry Weight - Test Tube (g)		0.3903	
Mass of PCL in Preparation (g)		0.5006	
Mass of FLT in Preparation (g)		0.0505	
Total (PCL +/- FLT) (g)		0.5511	
Percentage Yield (%)		70.82	

65kDa With FLT (PVA 13 -23kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6821	14.7914
	2	14.7403	14.8338
	3	14.7221	14.8201
	4	14.7260	14.8151
Total		58.8705	59.2604
Dry Weight - Test Tube (g)		0.3899	
Mass of PCL in Preparation (g)		0.5052	
Mass of FLT in Preparation (g)		0.0500	
Total (PCL +/- FLT) (g)		0.5552	
Percentage Yield (%)		70.22	

65kDa With FLT (PVA 13 - 23kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6754	14.7849
	2	14.7660	14.8719
	3	14.7658	14.8681
	4	14.6549	14.7267
Total		58.8621	59.2516
Dry Weight - Test Tube (g)		0.3895	
Mass of PCL in Preparation (g)		0.5000	
Mass of FLT in Preparation (g)		0.0501	
Total (PCL +/- FLT) (g)		0.5501	
Percentage Yield (%)		70.81	
Average Percentage Yield (%) (3 Sample)		70.62	

65kDa Without FLT (PVA 13 -23kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7594	14.8780
	2	14.7341	14.8141
	3	14.7194	14.8047
	4	14.6945	14.7730
Total		58.9074	59.2698
Dry Weight - Test Tube (g)		0.3624	
Mass of PCL in Preparation (g)		0.5005	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5005	
Percentage Yield (%)		72.41	

65kDa Without FLT (PVA 13 -23kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7885	14.8848
	2	14.6666	14.7500
	3	14.7980	14.8908
	4	14.6642	14.7531
Total		58.9173	59.2787
Dry Weight - Test Tube (g)		0.3614	
Mass of PCL in Preparation (g)		0.5016	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5016	
Percentage Yield (%)		72.05	

65kDa Without FLT (PVA 13 - 23kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6200	14.7010
	2	14.7625	14.8625
	3	14.6584	14.7534
	4	14.6480	14.6967
Total		58.6889	59.0136
Dry Weight - Test Tube (g)		0.3247	
Mass of PCL in Preparation (g)		0.5021	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5021	
Percentage Yield (%)		64.67	
Average Percentage Yield (%) (3 Sample)		69.71	

65kDa With FLT (PVA 30 - 70kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6842	14.7684
	2	14.6648	14.7701
	3	14.6381	14.7707
	4	14.7864	14.8669
Total		58.7735	59.1761
Dry Weight - Test Tube (g)		0.4026	
Mass of PCL in Preparation (g)		0.5011	
Mass of FLT in Preparation (g)		0.0504	
Total (PCL +/- FLT) (g)		0.5515	
Percentage Yield (%)		73.00	

65kDa With FLT (PVA 30 - 70kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6314	14.7404
	2	14.7168	14.8214
	3	14.7237	14.8119
	4	14.7660	14.8226
Total		58.8379	59.1963
Dry Weight - Test Tube (g)		0.3584	
Mass of PCL in Preparation (g)		0.5034	
Mass of FLT in Preparation (g)		0.0501	
Total (PCL +/- FLT) (g)		0.5535	
Percentage Yield (%)		64.75	

65kDa With FLT (PVA 30 - 70kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6754	14.7834
	2	14.7660	14.8912
	3	14.7658	14.8566
	4	14.6549	14.7234
Total		58.8621	59.2546
Dry Weight - Test Tube (g)		0.3925	
Mass of PCL in Preparation (g)		0.5015	
Mass of FLT in Preparation (g)		0.0500	
Total (PCL +/- FLT) (g)		0.5515	
Percentage Yield (%)		71.17	
Average Percentage Yield (%) (3 Sample)		69.64	

65kDa Without FLT (PVA 30 - 70kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6459	14.7305
	2	14.6113	14.7186
	3	14.6897	14.7535
	4	14.7945	14.8890
Total		58.7414	59.0916
Dry Weight - Test Tube (g)		0.3502	
Mass of PCL in Preparation (g)		0.5005	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5005	
Percentage Yield (%)		69.97	

65kDa Without FLT (PVA 30 - 70kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7684	14.8761
	2	14.7689	14.8360
	3	14.6289	14.7184
	4	14.7632	14.8409
Total		58.9294	59.2714
Dry Weight - Test Tube (g)		0.3420	
Mass of PCL in Preparation (g)		0.5016	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5016	
Percentage Yield (%)		68.18	

65kDa Without FLT (PVA 30 - 70kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6349	14.7016
	2	14.7713	14.8664
	3	14.7690	14.8462
	4	14.7541	14.8666
Total		58.9293	59.2808
Dry Weight - Test Tube (g)		0.3515	
Mass of PCL in Preparation (g)		0.5020	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5020	
Percentage Yield (%)		70.02	
Average Percentage Yield (%) (3 Sample)		69.39	

10kDa With FLT (PVA 13 - 23kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7613	14.8266
	2	14.6725	14.7625
	3	14.6984	14.8111
	4	14.7364	14.8180
Total		58.8686	59.2182
Dry Weight - Test Tube (g)		0.3496	
Mass of PCL in Preparation (g)		0.5009	
Mass of FLT in Preparation (g)		0.0249	
Total (PCL +/- FLT) (g)		0.5258	
Percentage Yield (%)		66.49	

10kDa With FLT (PVA 13 - 23kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6211	14.7116
	2	14.7391	14.8138
	3	14.7466	14.8228
	4	14.6347	14.7349
Total		58.7415	59.0831
Dry Weight - Test Tube (g)		0.3416	
Mass of PCL in Preparation (g)		0.5052	
Mass of FLT in Preparation (g)		0.0254	
Total (PCL +/- FLT) (g)		0.5306	
Percentage Yield (%)		64.38	

10kDa With FLT (PVA 13 - 23kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6335	14.7354
	2	14.7157	14.8119
	3	14.7100	14.7656
	4	14.7693	14.8617
Total		58.8285	59.1746
Dry Weight - Test Tube (g)		0.3461	
Mass of PCL in Preparation (g)		0.5000	
Mass of FLT in Preparation (g)		0.0250	
Total (PCL +/- FLT) (g)		0.5250	
Percentage Yield (%)		65.92	
Average Percentage Yield (%) (3 Sample)		65.60	

10kDa Without FLT (PVA 13 - 23kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.8018	14.8670
	2	14.6845	14.7762
	3	14.6448	14.7214
	4	14.6369	14.7222
Total		58.7680	59.0868
Dry Weight - Test Tube (g)		0.3188	
Mass of PCL in Preparation (g)		0.5021	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5021	
Percentage Yield (%)		63.49	

10kDa Without FLT (PVA 13 - 23kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7225	14.8125
	2	14.6674	14.7664
	3	14.7514	14.8400
	4	14.7162	14.7643
Total		58.8575	59.1832
Dry Weight - Test Tube (g)		0.3257	
Mass of PCL in Preparation (g)		0.5010	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5010	
Percentage Yield (%)		65.01	

10kDa Without FLT (PVA 13 - 23kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6399	14.7180
	2	14.7554	14.8350
	3	14.6753	14.7904
	4	14.6911	14.7501
Total		58.7617	59.0935
Dry Weight - Test Tube (g)		0.3318	
Mass of PCL in Preparation (g)		0.5003	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5003	
Percentage Yield (%)		66.32	
Average Percentage Yield (%) (3 Sample)		64.94	

10kDa With FLT (PVA 30 - 70kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6455	14.7305
	2	14.6487	14.7577
	3	14.6357	14.7243
	4	14.7114	14.7664
Total		58.6413	58.9789
Dry Weight - Test Tube (g)		0.3376	
Mass of PCL in Preparation (g)		0.5013	
Mass of FLT in Preparation (g)		0.0252	
Total (PCL +/- FLT) (g)		0.5265	
Percentage Yield (%)		64.12	

10kDa With FLT (PVA 30 - 70kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6821	14.7611
	2	14.7699	14.8602
	3	14.8116	14.8892
	4	14.6221	14.7224
Total		58.8857	59.2329
Dry Weight - Test Tube (g)		0.3472	
Mass of PCL in Preparation (g)		0.5028	
Mass of FLT in Preparation (g)		0.0249	
Total (PCL +/- FLT) (g)		0.5277	
Percentage Yield (%)		65.79	

10kDa With FLT (PVA 30 - 70kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6324	14.7256
	2	14.7691	14.8642
	3	14.7366	14.8064
	4	14.7225	14.8045
Total		58.8606	59.2007
Dry Weight - Test Tube (g)		0.3401	
Mass of PCL in Preparation (g)		0.5016	
Mass of FLT in Preparation (g)		0.0255	
Total (PCL +/- FLT) (g)		0.5271	
Percentage Yield (%)		64.52	
Average Percentage Yield (%) (3 Sample)		64.81	

10kDa Without FLT (PVA 30 - 70kDa)			
Sample 1	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6354	14.7149
	2	14.6954	14.7754
	3	14.6112	14.7060
	4	14.6475	14.7229
Total		58.5895	58.9192
Dry Weight - Test Tube (g)		0.3297	
Mass of PCL in Preparation (g)		0.5000	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5000	
Percentage Yield (%)		65.94	

10kDa Without FLT (PVA 30 - 70kDa)			
Sample 2	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.7330	14.8095
	2	14.6008	14.6881
	3	14.7156	14.7761
	4	14.6481	14.7443
Total		58.6975	59.0180
Dry Weight - Test Tube (g)		0.3205	
Mass of PCL in Preparation (g)		0.5015	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5015	
Percentage Yield (%)		63.91	

10kDa Without FLT (PVA 30 - 70kDa)			
Sample 3	**Test Tube**	**Empty Test Tube Weight (TT) (g)**	**Total Dry Weight (DW) (g)**
	1	14.6399	14.6973
	2	14.7554	14.8448
	3	14.6753	14.7510
	4	14.6911	14.7790
Total		58.7617	59.0721
Dry Weight - Test Tube (g)		0.3104	
Mass of PCL in Preparation (g)		0.5014	
Mass of FLT in Preparation (g)		0.0000	
Total (PCL +/- FLT) (g)		0.5014	
Percentage Yield (%)		61.91	
Average Percentage Yield (%) (3 Sample)		63.92	

Appendix : 3.5.1.

Mastersizer PCL 80kDa : PVA 13-23kDa (-FLT)

Analysis

Created by: Malvern Instruments Ltd
Last edited: 13/04/2012 16:12:51

Malvern

Measurement Details

Sample Name	PCL Microspheres without drug RI1.5 A0.001	**Measurement Date Time**	28/01/2014 14:57:32
Operator Name	CB	**Analysis Date Time**	28/01/2014 14:57:32
SOP File Name	HydroEV.cfg	**Result Source**	Measurement

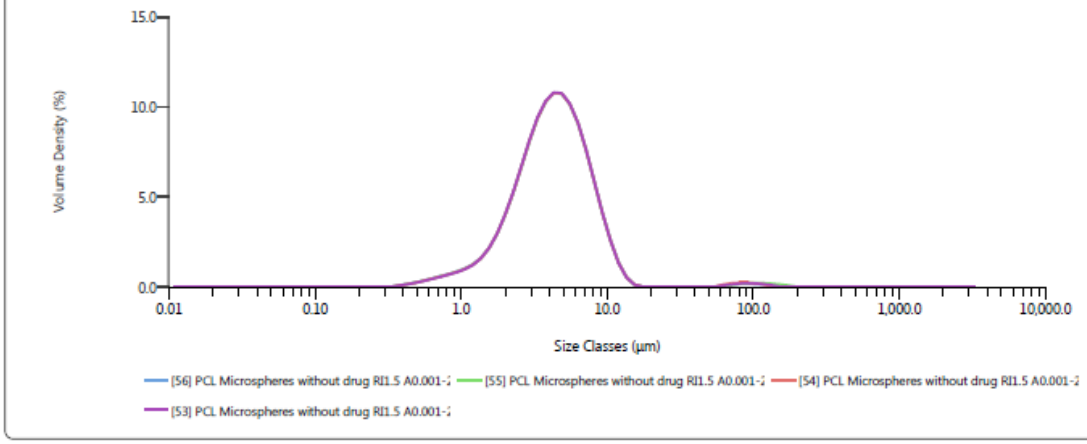
Analysis

Particle Name	Polycaprolacto	**Particle Refractive Index**	1.500
Dispersant Name	Water	**Dispersant Refractive Index**	1.330
Particle Absorption Index	0.001	**Laser Obscuration**	8.36 %
Weighted Residual	0.36 %	**Scattering Model**	Mie
Analysis Model	General Purpose	**Analysis Sensitivity**	Normal

Result

Concentration	0.0033 %	**Span**	1.508
Uniformity	0.610	**Result Units**	Volume
Specific Surface Area	1852 m^2/kg	**Dv (10)**	1.85 µm
D [3,2]	3.24 µm	**Dv (50)**	4.23 µm
D [4,3]	5.31 µm	**Dv (90)**	8.23 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.00	0.0679	0.00	0.460	0.21	3.12	7.82	21.2	0.00	144	0.00	976	0.00
0.0114	0.00	0.0771	0.00	0.523	0.29	3.55	8.61	24.1	0.00	163	0.00	1110	0.00
0.0129	0.00	0.0876	0.00	0.594	0.39	4.03	9.02	27.4	0.00	186	0.00	1260	0.00
0.0147	0.00	0.0995	0.00	0.675	0.49	4.58	8.99	31.1	0.00	211	0.00	1430	0.00
0.0167	0.00	0.113	0.00	0.767	0.59	5.21	8.51	35.3	0.00	240	0.00	1630	0.00
0.0189	0.00	0.128	0.00	0.872	0.70	5.92	7.61	40.1	0.00	272	0.00	1850	0.00
0.0215	0.00	0.146	0.00	0.991	0.84	6.72	6.36	45.6	0.00	310	0.00	2100	0.00
0.0244	0.00	0.166	0.00	1.13	1.03	7.64	4.92	51.8	0.00	352	0.00	2390	0.00
0.0278	0.00	0.188	0.00	1.28	1.32	8.68	3.46	58.9	0.07	400	0.00	2710	0.00
0.0315	0.00	0.214	0.00	1.45	1.77	9.86	2.15	66.9	0.11	454	0.00	3080	0.00
0.0358	0.00	0.243	0.00	1.65	2.43	11.2	1.10	76.0	0.14	516	0.00	3500	
0.0407	0.00	0.276	0.00	1.88	3.30	12.7	0.41	86.4	0.14	586	0.00		
0.0463	0.00	0.314	0.00	2.13	4.36	14.5	0.08	98.1	0.13	666	0.00		
0.0526	0.00	0.357	0.07	2.42	5.54	16.4	0.00	111	0.10	756	0.00		
0.0597	0.00	0.405	0.13	2.75	6.75	18.7	0.00	127	0.07	859	0.00		

Software Version: 2.20
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Mastersizer PCL 80kDa : PVA 13-23kDa (+FLT)

Analysis

Created by: DT
Last edited: 04/03/2014 15:53:33

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Measurement Details

Sample Name	B1	Measurement Date Time	04/09/2014 15:57:25
Operator Name	DT	Analysis Date Time	04/09/2014 15:57:25
SOP File Name	HydroEV.cfg	Result Source	Measurement

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	74	B1	0.0752	3.00	7.35
	75	B1	0.0797	3.04	7.59
	76	B1	0.0802	3.01	7.26
	77	B1	0.0808	3.03	7.44
	78	B1	0.0776	3.02	7.42
	79	Average of 'B1'	0.0786	3.02	7.41
Mean			**0.0787**	**3.02**	**7.41**
1xStd Dev			**0.00204**	**0.0164**	**0.110**
1xRSD (%)			**2.59**	**0.541**	**1.49**

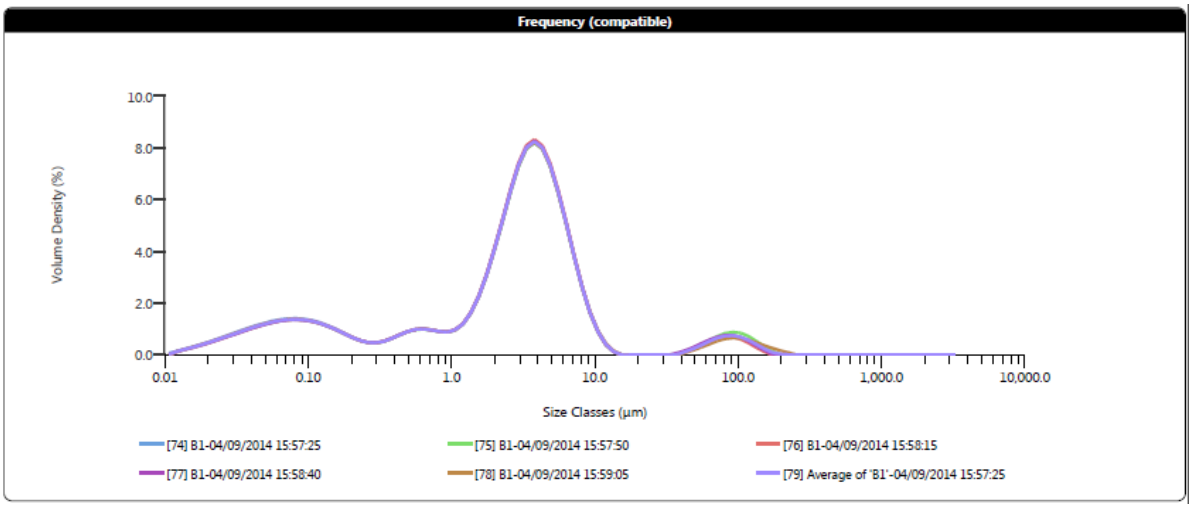
Analysis

Particle Name	Polycaprolacto	Particle Refractive Index	1.500
Dispersant Name	Water	Dispersant Refractive Index	1.330
Particle Absorption Index	0.001	Laser Obscuration	6.01 %
Weighted Residual	0.67 %	Scattering Model	Mie
Analysis Model	General Purpose	Analysis Sensitivity	Normal

Result

Concentration	0.0026 %	Span	2.426
Uniformity	1.882	Result Units	Volume
Specific Surface Area	24060 m²/kg	Dv (10)	0.0752 µm
D [3,2]	0.249 µm	Dv (50)	3.00 µm
D [4,3]	6.82 µm	Dv (90)	7.35 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.06	0.0679	1.17	0.460	0.75	3.12	6.68	21.2	0.00	144	0.15	976	0.00
0.0114	0.12	0.0771	1.18	0.523	0.83	3.55	6.88	24.1	0.00	163	0.00	1110	0.00
0.0129	0.19	0.0876	1.16	0.594	0.85	4.03	6.67	27.4	0.00	186	0.00	1260	0.00
0.0147	0.26	0.0995	1.12	0.675	0.81	4.58	6.10	31.1	0.00	211	0.00	1430	0.00
0.0167	0.34	0.113	1.05	0.767	0.76	5.21	5.25	35.3	0.00	240	0.00	1630	0.00
0.0189	0.42	0.128	0.95	0.872	0.73	5.92	4.22	40.1	0.11	272	0.00	1850	0.00
0.0215	0.51	0.146	0.84	0.991	0.79	6.72	3.16	45.6	0.21	310	0.00	2100	0.00
0.0244	0.60	0.166	0.71	1.13	0.98	7.64	2.17	51.8	0.33	352	0.00	2390	0.00
0.0278	0.69	0.188	0.58	1.28	1.34	8.68	1.34	58.9	0.46	400	0.00	2710	0.00
0.0315	0.78	0.214	0.47	1.45	1.86	9.86	0.72	66.9	0.56	454	0.00	3080	0.00
0.0358	0.87	0.243	0.40	1.65	2.56	11.2	0.31	76.0	0.63	516	0.00	3500	
0.0407	0.95	0.276	0.38	1.88	3.39	12.7	0.09	86.4	0.64	586	0.00		
0.0463	1.03	0.314	0.43	2.13	4.32	14.5	0.00	98.1	0.57	666	0.00		
0.0526	1.09	0.357	0.52	2.42	5.27	16.4	0.00	111	0.45	756	0.00		
0.0597	1.14	0.405	0.64	2.75	6.11	18.7	0.00	127	0.30	859	0.00		

Mastersizer PCL 80kDa : PVA 30-70kDa (-FLT)

Analysis

Created by: Malvern Instruments Ltd
Last edited: 13/04/2012 16:12:51

Malvern

Measurement Details

Sample Name	Average of '80K 30-70 without drug'	Measurement Date Time	12/02/2014 14:35:50
Operator Name	CB	Analysis Date Time	12/02/2014 14:35:50
SOP File Name	HydroEV.cfg	Result Source	Averaged

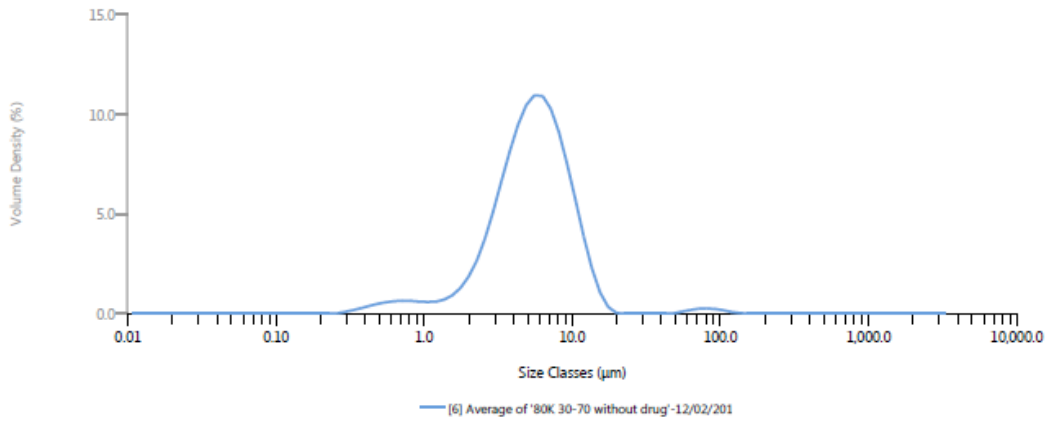
Analysis

Particle Name	Polycaprolacto	Particle Refractive Index	1.500
Dispersant Name	Water	Dispersant Refractive Index	1.330
Particle Absorption Index	0.001	Laser Obscuration	7.04 %
Weighted Residual	0.46 %	Scattering Model	Mie
Analysis Model	General Purpose	Analysis Sensitivity	Enhanced

Result

Concentration	0.0036 %	Span	1.495
Uniformity	0.593	Result Units	Volume
Specific Surface Area	1635 m^2/kg	Dv (10)	2.31 µm
D [3,2]	3.67 µm	Dv (50)	5.41 µm
D [4,3]	6.64 µm	Dv (90)	10.4 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.00	0.0679	0.00	0.460	0.41	3.12	5.54	21.2	0.00	144	0.00	976	0.00
0.0114	0.00	0.0771	0.00	0.523	0.48	3.55	6.80	24.1	0.00	163	0.00	1110	0.00
0.0129	0.00	0.0876	0.00	0.594	0.52	4.03	7.92	27.4	0.00	186	0.00	1260	0.00
0.0147	0.00	0.0995	0.00	0.675	0.54	4.58	8.74	31.1	0.00	211	0.00	1430	0.00
0.0167	0.00	0.113	0.00	0.767	0.52	5.21	9.17	35.3	0.00	240	0.00	1630	0.00
0.0189	0.00	0.128	0.00	0.872	0.50	5.92	9.14	40.1	0.00	272	0.00	1850	0.00
0.0215	0.00	0.146	0.00	0.991	0.48	6.72	8.61	45.6	0.00	310	0.00	2100	0.00
0.0244	0.00	0.166	0.00	1.13	0.50	7.64	7.61	51.8	0.09	352	0.00	2390	0.00
0.0278	0.00	0.188	0.00	1.28	0.57	8.68	6.27	58.9	0.15	400	0.00	2710	0.00
0.0315	0.00	0.214	0.00	1.45	0.75	9.86	4.74	66.9	0.20	454	0.00	3080	0.00
0.0358	0.00	0.243	0.00	1.65	1.05	11.2	3.21	76.0	0.21	516	0.00	3500	
0.0407	0.00	0.276	0.08	1.88	1.52	12.7	1.87	86.4	0.19	586	0.00		
0.0463	0.00	0.314	0.15	2.13	2.21	14.5	0.85	98.1	0.13	666	0.00		
0.0526	0.00	0.357	0.23	2.42	3.13	16.4	0.24	111	0.06	756	0.00		
0.0597	0.00	0.405	0.32	2.75	4.27	18.7	0.00	127	0.01	859	0.00		

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Mastersizer PCL 80kDa : PVA 30-70kDa (+FLT)

Analysis

Created by: Malvern Instruments Ltd
Last edited: 13/04/2012 16:12:51

Malvern

Measurement Details

Sample Name	Average of '80K 30-70 with drug'	Measurement Date Time	12/02/2014 14:50:10
Operator Name	CB	Analysis Date Time	12/02/2014 14:50:10
SOP File Name	HydroEV.cfg	Result Source	Averaged

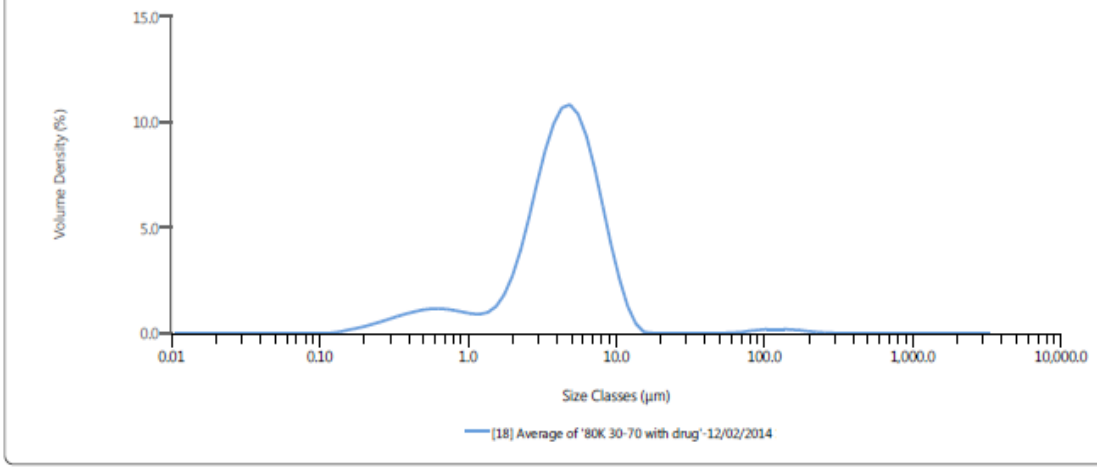
Analysis

Particle Name	Polycaprolacto	Particle Refractive Index	1.500
Dispersant Name	Water	Dispersant Refractive Index	1.330
Particle Absorption Index	0.001	Laser Obscuration	6.81 %
Weighted Residual	0.43 %	Scattering Model	Mie
Analysis Model	General Purpose	Analysis Sensitivity	Enhanced

Result

Concentration	0.0029 %	Span	1.684
Uniformity	0.780	Result Units	Volume
Specific Surface Area	2740 m²/kg	Dv (10)	1.05 µm
D [3,2]	2.19 µm	Dv (50)	4.28 µm
D [4,3]	5.85 µm	Dv (90)	8.25 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.00	0.0679	0.00	0.460	0.91	3.12	7.25	21.2	0.00	144	0.13	976	0.00
0.0114	0.00	0.0771	0.00	0.523	0.95	3.55	8.30	24.1	0.00	163	0.10	1110	0.00
0.0129	0.00	0.0876	0.00	0.594	0.96	4.03	8.94	27.4	0.00	186	0.06	1260	0.00
0.0147	0.00	0.0995	0.00	0.675	0.94	4.58	9.07	31.1	0.00	211	0.04	1430	0.00
0.0167	0.00	0.113	0.00	0.767	0.89	5.21	8.69	35.3	0.00	240	0.02	1630	0.00
0.0189	0.00	0.128	0.04	0.872	0.82	5.92	7.81	40.1	0.00	272	0.00	1850	0.00
0.0215	0.00	0.146	0.11	0.991	0.76	6.72	6.52	45.6	0.00	310	0.00	2100	0.00
0.0244	0.00	0.166	0.18	1.13	0.73	7.64	5.02	51.8	0.00	352	0.00	2390	0.00
0.0278	0.00	0.188	0.26	1.28	0.80	8.68	3.49	58.9	0.01	400	0.00	2710	0.00
0.0315	0.00	0.214	0.35	1.45	1.03	9.86	2.11	66.9	0.03	454	0.00	3080	0.00
0.0358	0.00	0.243	0.44	1.65	1.48	11.2	1.04	76.0	0.09	516	0.00	3500	
0.0407	0.00	0.276	0.55	1.88	2.23	12.7	0.35	86.4	0.12	586	0.00		
0.0463	0.00	0.314	0.65	2.13	3.26	14.5	0.00	98.1	0.14	666	0.00		
0.0526	0.00	0.357	0.75	2.42	4.53	16.4	0.00	111	0.15	756	0.00		
0.0597	0.00	0.405	0.84	2.75	5.93	18.7	0.00	127	0.15	859	0.00		

Software Version: 2.20
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Mastersizer PCL 65kDa : PVA 13-23kDa (-FLT)

Analysis

Created by: DT
Last edited: 04/03/2014 15:53:33

Malvern

Measurement Details

Sample Name	C10_5	Measurement Date Time	19/08/2014 09:40:11
Operator Name	DT	Analysis Date Time	19/08/2014 09:40:11
SOP File Name	HydroEV.cfg	Result Source	Measurement

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	13	C10_5	0.0747	4.35	8.63
	14	C10_5	0.0669	4.25	8.61
	15	C10_5	0.0681	4.26	8.63
	16	C10_5	0.0680	4.26	8.64
	17	C10_5	0.0667	4.25	8.56
	18	Average of 'C10_5'	0.0687	4.27	8.61
Mean			**0.0688**	**4.27**	**8.61**
1xStd Dev			**0.00297**	**0.0366**	**0.0275**
1xRSD (%)			**4.32**	**0.856**	**0.319**

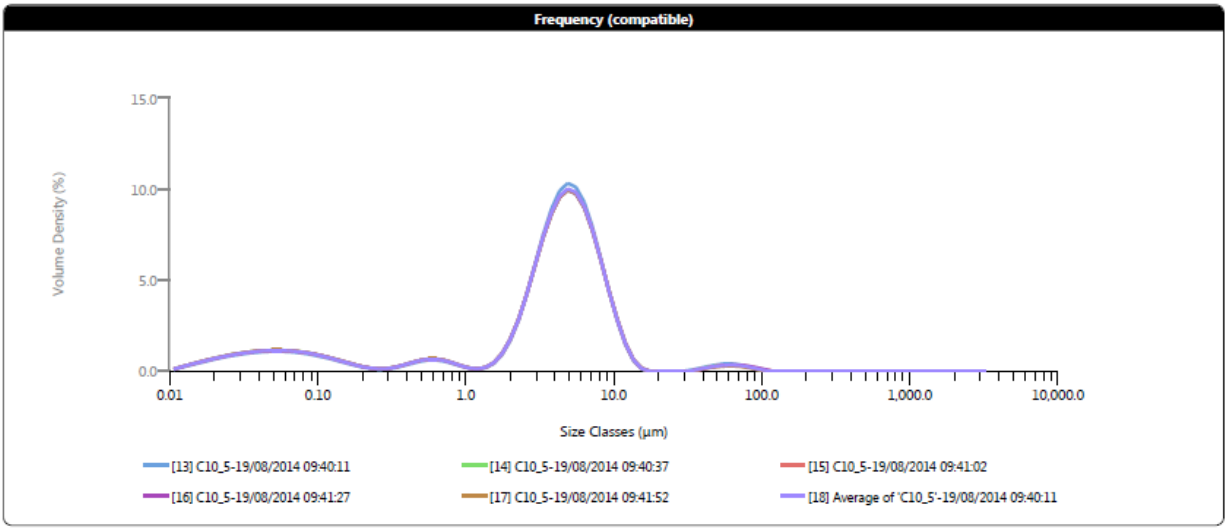
Analysis

Particle Name	Polycaprolacto	Particle Refractive Index	1.500
Dispersant Name	Water	Dispersant Refractive Index	1.330
Particle Absorption Index	0.001	Laser Obscuration	7.20 %
Weighted Residual	0.59 %	Scattering Model	Mie
Analysis Model	General Purpose	Analysis Sensitivity	Normal

Result

Concentration	0.0041 %	Span	1.969
Uniformity	0.775	Result Units	Volume
Specific Surface Area	23640 m²/kg	Dv (10)	0.0747 µm
D [3,2]	0.254 µm	Dv (50)	4.35 µm
D [4,3]	5.49 µm	Dv (90)	8.63 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.09	0.0679	0.86	0.460	0.46	3.12	6.26	21.2	0.00	144	0.00	976	0.00
0.0114	0.19	0.0771	0.81	0.523	0.52	3.55	7.46	24.1	0.00	163	0.00	1110	0.00
0.0129	0.28	0.0876	0.75	0.594	0.52	4.03	8.30	27.4	0.00	186	0.00	1260	0.00
0.0147	0.38	0.0995	0.67	0.675	0.46	4.58	8.65	31.1	0.06	211	0.00	1430	0.00
0.0167	0.47	0.113	0.58	0.767	0.35	5.21	8.48	35.3	0.13	240	0.00	1630	0.00
0.0189	0.55	0.128	0.48	0.872	0.23	5.92	7.78	40.1	0.22	272	0.00	1850	0.00
0.0215	0.63	0.146	0.37	0.991	0.13	6.72	6.64	45.6	0.30	310	0.00	2100	0.00
0.0244	0.71	0.166	0.27	1.13	0.09	7.64	5.21	51.8	0.35	352	0.00	2390	0.00
0.0278	0.77	0.188	0.19	1.28	0.15	8.68	3.71	58.9	0.35	400	0.00	2710	0.00
0.0315	0.82	0.214	0.12	1.45	0.34	9.86	2.32	66.9	0.30	454	0.00	3080	0.00
0.0358	0.86	0.243	0.09	1.65	0.73	11.2	1.20	76.0	0.21	516	0.00	3500	
0.0407	0.89	0.276	0.11	1.88	1.37	12.7	0.46	86.4	0.10	586	0.00		
0.0463	0.91	0.314	0.16	2.13	2.30	14.5	0.09	98.1	0.00	666	0.00		
0.0526	0.91	0.357	0.26	2.42	3.50	16.4	0.00	111	0.00	756	0.00		
0.0597	0.89	0.405	0.36	2.75	4.87	18.7	0.00	127	0.00	859	0.00		

Mastersizer PCL 65kDa : PVA 13-23kDa (+FLT)

Analysis

Created by: DT
Last edited: 04/03/2014 15:53:33

Malvern

Measurement Details

Sample Name	Batch-C7	**Measurement Date Time**	31/07/2014 09:25:04
Operator Name	DT	**Analysis Date Time**	31/07/2014 09:25:04
SOP File Name	HydroEV.cfg	**Result Source**	Measurement

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	Batch-C7	0.0371	3.34	7.90
	2	Batch-C7	0.0366	3.30	7.66
	3	Batch-C7	0.0364	3.30	7.49
	4	Batch-C7	0.0364	3.28	7.57
	5	Batch-C7	0.0365	3.29	7.65
	6	Average of 'Batch-C7'	0.0366	3.30	7.64
Mean			**0.0366**	**3.30**	**7.65**
1xStd Dev			**0.000281**	**0.0186**	**0.139**
1xRSD (%)			**0.768**	**0.564**	**1.82**

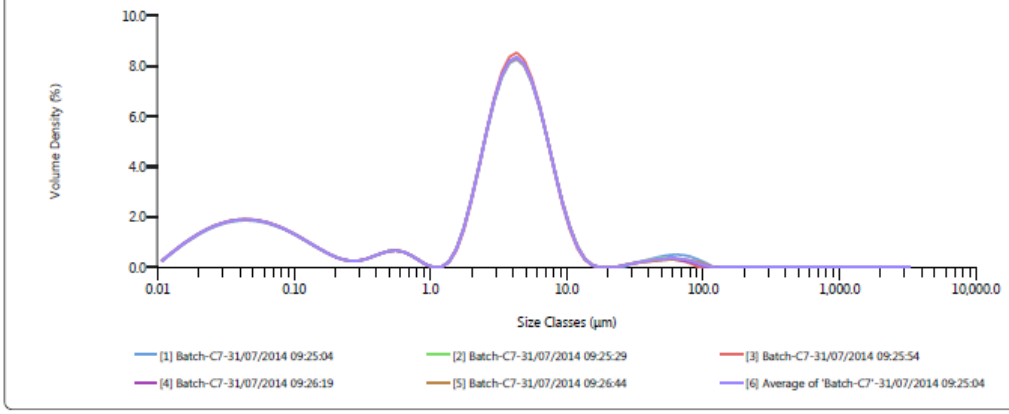
Analysis

Particle Name	Polycaprolacto	**Particle Refractive Index**	1.500
Dispersant Name	Water	**Dispersant Refractive Index**	1.330
Particle Absorption Index	0.001	**Laser Obscuration**	7.05 %
Weighted Residual	0.61 %	**Scattering Model**	Mie
Analysis Model	General Purpose	**Analysis Sensitivity**	Normal

Result

Concentration	0.0039 %	**Span**	2.356
Uniformity	1.216	**Result Units**	Volume
Specific Surface Area	42940 m²/kg	**Dv (10)**	0.0371 µm
D [3,2]	0.140 µm	**Dv (50)**	3.34 µm
D [4,3]	5.21 µm	**Dv (90)**	7.90 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.21	0.0679	1.38	0.460	0.54	3.12	6.30	21.2	0.00	144	0.00	976	0.00
0.0114	0.41	0.0771	1.28	0.523	0.57	3.55	6.79	24.1	0.00	163	0.00	1110	0.00
0.0129	0.60	0.0876	1.16	0.594	0.52	4.03	6.92	27.4	0.00	186	0.00	1260	0.00
0.0147	0.78	0.0995	1.03	0.675	0.40	4.58	6.69	31.1	0.16	211	0.00	1430	0.00
0.0167	0.95	0.113	0.89	0.767	0.24	5.21	6.14	35.3	0.23	240	0.00	1630	0.00
0.0189	1.10	0.128	0.74	0.872	0.09	5.92	5.30	40.1	0.29	272	0.00	1850	0.00
0.0215	1.24	0.146	0.59	0.991	0.00	6.72	4.27	45.6	0.36	310	0.00	2100	0.00
0.0244	1.35	0.166	0.46	1.13	0.00	7.64	3.19	51.8	0.41	352	0.00	2390	0.00
0.0278	1.44	0.188	0.34	1.28	0.16	8.68	2.17	58.9	0.43	400	0.00	2710	0.00
0.0315	1.50	0.214	0.25	1.45	0.58	9.86	1.32	66.9	0.42	454	0.00	3080	0.00
0.0358	1.54	0.243	0.21	1.65	1.28	11.2	0.68	76.0	0.36	516	0.00	3500	
0.0407	1.56	0.276	0.22	1.88	2.23	12.7	0.27	86.4	0.26	586	0.00		
0.0463	1.55	0.314	0.27	2.13	3.33	14.5	0.06	98.1	0.15	666	0.00		
0.0526	1.52	0.357	0.36	2.42	4.47	16.4	0.00	111	0.00	756	0.00		
0.0597	1.46	0.405	0.46	2.75	5.50	18.7	0.00	127	0.00	859	0.00		

Mastersizer PCL 65kDa : PVA 30-70kDa (-FLT)

Analysis

Created by: DT
Last edited: 04/03/2014 15:53:33

Malvern

Measurement Details

Sample Name	D10_4	**Measurement Date Time**	19/08/2014 10:00:39
Operator Name	DT	**Analysis Date Time**	19/08/2014 10:00:39
SOP File Name	HydroEV.cfg	**Result Source**	Measurement

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	31	D10_4	2.88	5.97	10.9
	32	D10_4	2.88	5.99	10.9
	33	D10_4	2.88	5.96	10.8
	34	D10_4	2.88	5.97	10.8
	35	D10_4	2.88	5.95	10.7
	36	Average of 'D10_4'	2.88	5.97	10.8
Mean			**2.88**	**5.97**	**10.8**
1xStd Dev			**0.00211**	**0.0110**	**0.0595**
1xRSD (%)			**0.0734**	**0.184**	**0.549**

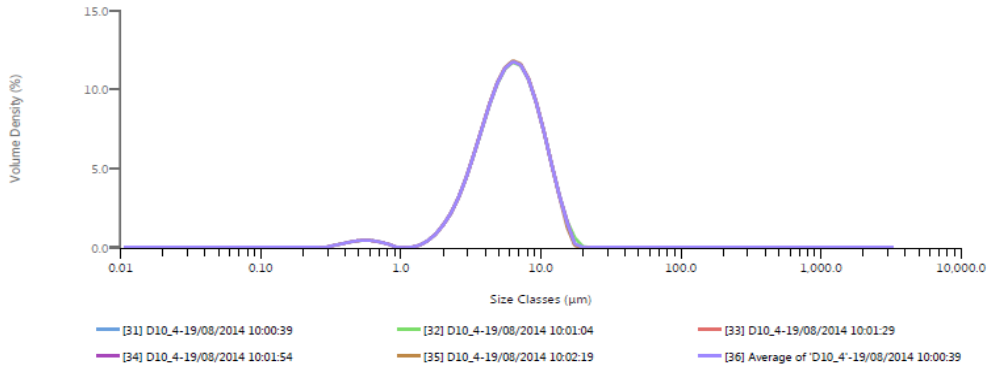
Analysis

Particle Name	Polycaprolacto	**Particle Refractive Index**	1.500
Dispersant Name	Water	**Dispersant Refractive Index**	1.330
Particle Absorption Index	0.001	**Laser Obscuration**	6.95 %
Weighted Residual	0.65 %	**Scattering Model**	Mie
Analysis Model	General Purpose	**Analysis Sensitivity**	Normal

Result

Concentration	0.0041 %	**Span**	1.335
Uniformity	0.413	**Result Units**	Volume
Specific Surface Area	1363 m^2/kg	**Dv (10)**	2.88 µm
D [3,2]	4.40 µm	**Dv (50)**	5.97 µm
D [4,3]	6.44 µm	**Dv (90)**	10.9 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.00	0.0679	0.00	0.460	0.35	3.12	4.90	21.2	0.00	144	0.00	976	0.00
0.0114	0.00	0.0771	0.00	0.523	0.38	3.55	6.25	24.1	0.00	163	0.00	1110	0.00
0.0129	0.00	0.0876	0.00	0.594	0.35	4.03	7.56	27.4	0.00	186	0.00	1260	0.00
0.0147	0.00	0.0995	0.00	0.675	0.28	4.58	8.68	31.1	0.00	211	0.00	1430	0.00
0.0167	0.00	0.113	0.00	0.767	0.17	5.21	9.47	35.3	0.00	240	0.00	1630	0.00
0.0189	0.00	0.128	0.00	0.872	0.00	5.92	9.84	40.1	0.00	272	0.00	1850	0.00
0.0215	0.00	0.146	0.00	0.991	0.00	6.72	9.67	45.6	0.00	310	0.00	2100	0.00
0.0244	0.00	0.166	0.00	1.13	0.00	7.64	8.94	51.8	0.00	352	0.00	2390	0.00
0.0278	0.00	0.188	0.00	1.28	0.11	8.68	7.71	58.9	0.00	400	0.00	2710	0.00
0.0315	0.00	0.214	0.00	1.45	0.32	9.86	6.12	66.9	0.00	454	0.00	3080	0.00
0.0358	0.00	0.243	0.00	1.65	0.66	11.2	4.37	76.0	0.00	516	0.00	3500	
0.0407	0.00	0.276	0.00	1.88	1.14	12.7	2.72	86.4	0.00	586	0.00		
0.0463	0.00	0.314	0.10	2.13	1.78	14.5	1.37	98.1	0.00	666	0.00		
0.0526	0.00	0.357	0.19	2.42	2.61	16.4	0.02	111	0.00	756	0.00		
0.0597	0.00	0.405	0.28	2.75	3.66	18.7	0.00	127	0.00	859	0.00		

Mastersizer PCL 65kDa : PVA 30-70kDa (+FLT)

Analysis

Created by: Malvern Instruments Ltd
Last edited: 13/04/2012 16:12:51

Malvern

Measurement Details

Sample Name	Average of '65K with drug'	**Measurement Date Time**	04/02/2014 14:34:34
Operator Name	CB	**Analysis Date Time**	04/02/2014 14:34:34
SOP File Name	HydroEV.cfg	**Result Source**	Averaged

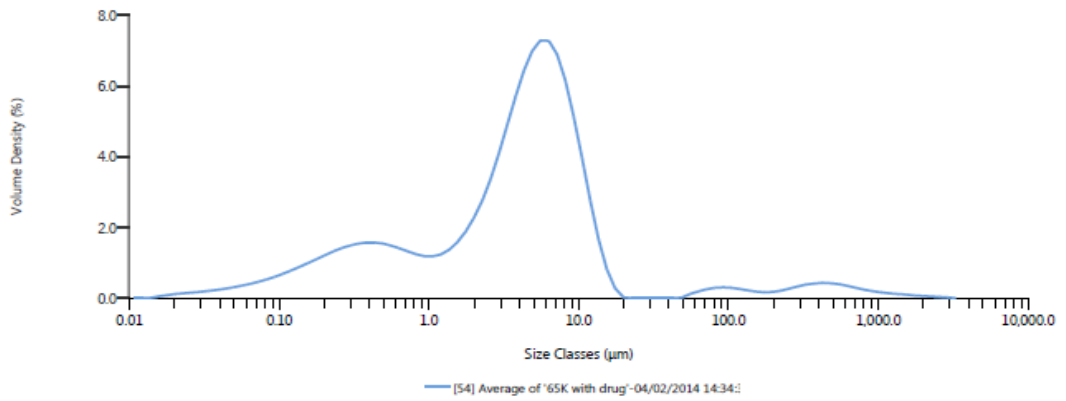
Analysis

Particle Name	Polycaprolacto	**Particle Refractive Index**	1.500
Dispersant Name	Water	**Dispersant Refractive Index**	1.330
Particle Absorption Index	0.001	**Laser Obscuration**	5.85 %
Weighted Residual	0.34 %	**Scattering Model**	Mie
Analysis Model	General Purpose	**Analysis Sensitivity**	Enhanced

Result

Concentration	0.0030 %	**Span**	2.628
Uniformity	7.045	**Result Units**	Volume
Specific Surface Area	9411 m^2/kg	**Dv (10)**	0.266 µm
D [3,2]	0.638 µm	**Dv (50)**	4.27 µm
D [4,3]	31.8 µm	**Dv (90)**	11.5 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.00	0.0679	0.38	0.460	1.29	3.12	4.08	21.2	0.00	144	0.15	976	0.14
0.0114	0.00	0.0771	0.44	0.523	1.24	3.55	4.76	24.1	0.00	163	0.13	1110	0.11
0.0129	0.00	0.0876	0.50	0.594	1.17	4.03	5.38	27.4	0.00	186	0.15	1260	0.10
0.0147	0.04	0.0995	0.57	0.675	1.10	4.58	5.85	31.1	0.00	211	0.18	1430	0.08
0.0167	0.07	0.113	0.65	0.767	1.03	5.21	6.10	35.3	0.00	240	0.22	1630	0.06
0.0189	0.09	0.128	0.74	0.872	0.98	5.92	6.09	40.1	0.00	272	0.28	1850	0.05
0.0215	0.11	0.146	0.83	0.991	0.98	6.72	5.77	45.6	0.00	310	0.32	2100	0.04
0.0244	0.13	0.166	0.92	1.13	1.03	7.64	5.15	51.8	0.10	352	0.35	2390	0.03
0.0278	0.15	0.188	1.01	1.28	1.14	8.68	4.30	58.9	0.16	400	0.36	2710	0.02
0.0315	0.17	0.214	1.10	1.45	1.31	9.86	3.31	66.9	0.21	454	0.35	3080	0.00
0.0358	0.19	0.243	1.17	1.65	1.56	11.2	2.28	76.0	0.25	516	0.33	3500	
0.0407	0.22	0.276	1.24	1.88	1.89	12.7	1.37	86.4	0.26	586	0.29		
0.0463	0.25	0.314	1.29	2.13	2.30	14.5	0.66	98.1	0.24	666	0.24		
0.0526	0.29	0.357	1.31	2.42	2.81	16.4	0.21	111	0.21	756	0.20		
0.0597	0.33	0.405	1.31	2.75	3.41	18.7	0.00	127	0.18	859	0.16		

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Mastersizer PCL 10kDa : PVA 13-23kDa (-FLT)

Analysis

Created by: DT
Last edited: 04/03/2014 15:53:33

Malvern

Measurement Details

Sample Name	A10_SSC	Measurement Date Time	12/08/2014 09:43:10
Operator Name	DT	Analysis Date Time	12/08/2014 09:43:10
SOP File Name	HydroEV.cfg	Result Source	Measurement

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	A10_SSC	1.88	3.84	8.39
	2	A10_SSC	1.88	3.86	8.57
	3	A10_SSC	1.88	3.86	8.59
	4	A10_SSC	1.89	3.87	8.72
	5	A10_SSC	1.89	3.90	9.17
	6	Average of 'A10_SSC'	1.88	3.87	8.65
Mean			**1.88**	**3.87**	**8.68**
1xStd Dev			**0.00379**	**0.0192**	**0.262**
1xRSD (%)			**0.201**	**0.497**	**3.02**

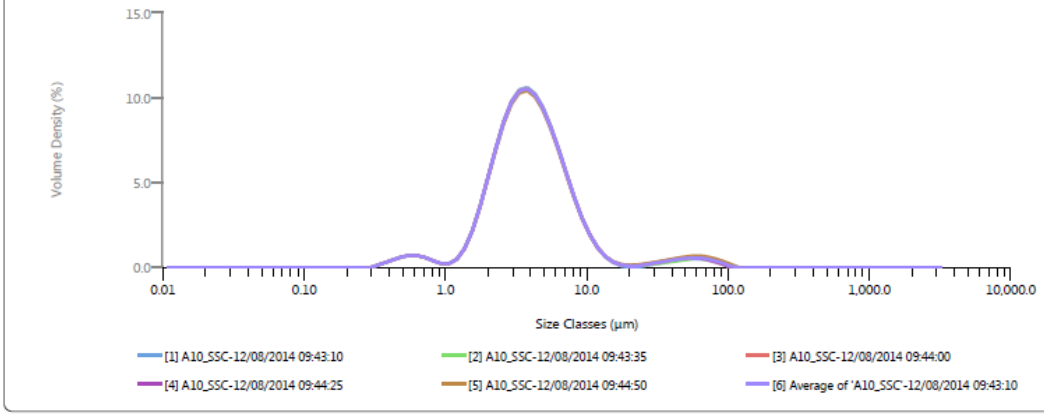
Analysis

Particle Name	Polycaprolacto	Particle Refractive Index	1.500
Dispersant Name	Water	Dispersant Refractive Index	1.330
Particle Absorption Index	0.001	Laser Obscuration	5.95 %
Weighted Residual	0.54 %	Scattering Model	Mie
Analysis Model	General Purpose	Analysis Sensitivity	Normal

Result

Concentration	0.0022 %	Span	1.695
Uniformity	0.886	Result Units	Volume
Specific Surface Area	2010 m²/kg	Dv (10)	1.88 µm
D [3,2]	2.98 µm	Dv (50)	3.84 µm
D [4,3]	5.95 µm	Dv (90)	8.39 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.00	0.0679	0.00	0.460	0.54	3.12	8.78	21.2	0.00	144	0.00	976	0.00
0.0114	0.00	0.0771	0.00	0.523	0.61	3.55	8.89	24.1	0.09	163	0.00	1110	0.00
0.0129	0.00	0.0876	0.00	0.594	0.60	4.03	8.58	27.4	0.16	186	0.00	1260	0.00
0.0147	0.00	0.0995	0.00	0.675	0.50	4.58	7.92	31.1	0.23	211	0.00	1430	0.00
0.0167	0.00	0.113	0.00	0.767	0.34	5.21	6.99	35.3	0.30	240	0.00	1630	0.00
0.0189	0.00	0.128	0.00	0.872	0.20	5.92	5.87	40.1	0.37	272	0.00	1850	0.00
0.0215	0.00	0.146	0.00	0.991	0.18	6.72	4.67	45.6	0.42	310	0.00	2100	0.00
0.0244	0.00	0.166	0.00	1.13	0.39	7.64	3.50	51.8	0.45	352	0.00	2390	0.00
0.0278	0.00	0.188	0.00	1.28	0.92	8.68	2.45	58.9	0.43	400	0.00	2710	0.00
0.0315	0.00	0.214	0.00	1.45	1.81	9.86	1.60	66.9	0.38	454	0.00	3080	0.00
0.0358	0.00	0.243	0.00	1.65	3.02	11.2	0.95	76.0	0.28	516	0.00	3500	
0.0407	0.00	0.276	0.00	1.88	4.44	12.7	0.51	86.4	0.16	586	0.00		
0.0463	0.00	0.314	0.13	2.13	5.91	14.5	0.23	98.1	0.00	666	0.00		
0.0526	0.00	0.357	0.26	2.42	7.22	16.4	0.08	111	0.00	756	0.00		
0.0597	0.00	0.405	0.41	2.75	8.21	18.7	0.00	127	0.00	859	0.00		

Mastersizer PCL 10kDa : PVA 13-23kDa (+FLT)

Analysis

Created by: DT
Last edited: 04/03/2014 15:53:33

Malvern

Measurement Details

Sample Name	Batch-A2	Measurement Date Time	29/07/2014 09:16:11
Operator Name	DT	Analysis Date Time	29/07/2014 09:16:11
SOP File Name	HydroEV.cfg	Result Source	Measurement

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	1	Batch-A2	0.0387	3.43	8.20
	2	Batch-A2	0.0385	3.43	8.28
	3	Batch-A2	0.0384	3.42	8.18
	4	Batch-A2	0.0381	3.40	8.06
	5	Batch-A2	0.0382	3.42	8.23
	6	Average of 'Batch-A2'	0.0384	3.42	8.19
Mean			**0.0384**	**3.42**	**8.19**
1xStd Dev			**0.000206**	**0.0105**	**0.0725**
1xRSD (%)			**0.536**	**0.307**	**0.885**

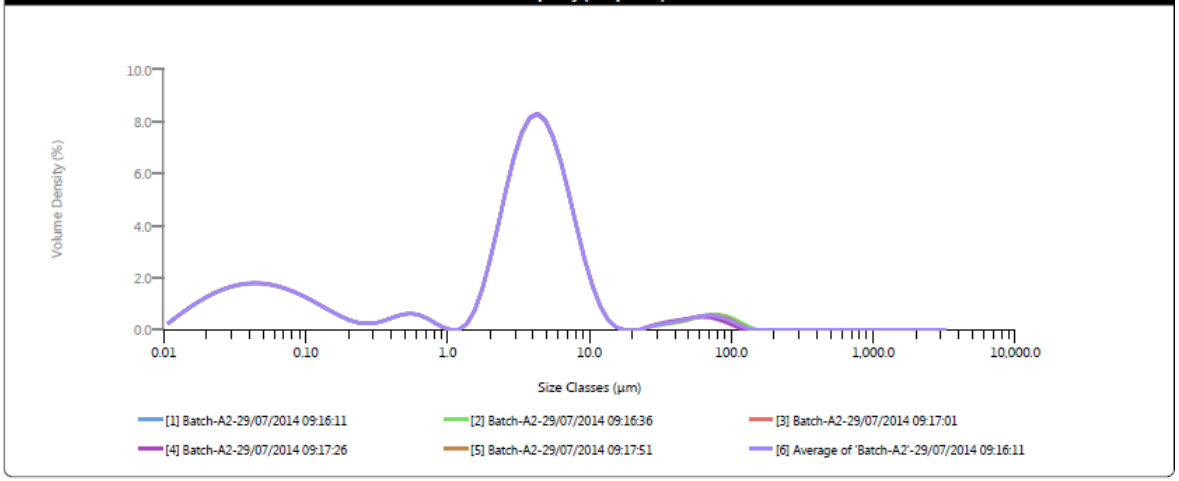
Analysis

Particle Name	Polycaprolacto	Particle Refractive Index	1.500
Dispersant Name	Water	Dispersant Refractive Index	1.330
Particle Absorption Index	0.001	Laser Obscuration	6.79 %
Weighted Residual	0.59 %	Scattering Model	Mie
Analysis Model	General Purpose	Analysis Sensitivity	Normal

Result

Concentration	0.0038 %	Span	2.380
Uniformity	1.330	Result Units	Volume
Specific Surface Area	41000 m²/kg	Dv (10)	0.0387 µm
D [3,2]	0.146 µm	Dv (50)	3.43 µm
D [4,3]	5.80 µm	Dv (90)	8.20 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.20	0.0679	1.31	0.460	0.51	3.12	6.33	21.2	0.00	144	0.00	976	0.00
0.0114	0.39	0.0771	1.22	0.523	0.53	3.55	6.82	24.1	0.08	163	0.00	1110	0.00
0.0129	0.57	0.0876	1.11	0.594	0.49	4.03	6.96	27.4	0.14	186	0.00	1260	0.00
0.0147	0.75	0.0995	0.98	0.675	0.37	4.58	6.74	31.1	0.19	211	0.00	1430	0.00
0.0167	0.91	0.113	0.84	0.767	0.22	5.21	6.20	35.3	0.23	240	0.00	1630	0.00
0.0189	1.05	0.128	0.70	0.872	0.08	5.92	5.37	40.1	0.28	272	0.00	1850	0.00
0.0215	1.18	0.146	0.56	0.991	0.00	6.72	4.35	45.6	0.34	310	0.00	2100	0.00
0.0244	1.29	0.166	0.43	1.13	0.00	7.64	3.27	51.8	0.41	352	0.00	2390	0.00
0.0278	1.37	0.188	0.32	1.28	0.17	8.68	2.25	58.9	0.47	400	0.00	2710	0.00
0.0315	1.43	0.214	0.24	1.45	0.59	9.86	1.38	66.9	0.49	454	0.00	3080	0.00
0.0358	1.47	0.243	0.19	1.65	1.29	11.2	0.72	76.0	0.46	516	0.00	3500	
0.0407	1.49	0.276	0.20	1.88	2.24	12.7	0.29	86.4	0.37	586	0.00		
0.0463	1.48	0.314	0.25	2.13	3.36	14.5	0.07	98.1	0.25	666	0.00		
0.0526	1.44	0.357	0.34	2.42	4.50	16.4	0.00	111	0.12	756	0.00		
0.0597	1.39	0.405	0.44	2.75	5.53	18.7	0.00	127	0.00	859	0.00		

Mastersizer PCL 10kDa : PVA 30-70kDa (-FLT)

Analysis

Created by: DT
Last edited: 04/08/2014 15:53:33

Malvern

Measurement Details

Sample Name	B10_SSC	Measurement Date Time	12/08/2014 10:25:26
Operator Name	DT	Analysis Date Time	12/08/2014 10:25:26
SOP File Name	HydroEV.cfg	Result Source	Measurement

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	37	B10_SSC	0.0345	3.25	6.87
	38	B10_SSC	0.0342	3.23	6.78
	39	B10_SSC	0.0342	3.23	6.82
	40	B10_SSC	0.0338	3.18	6.69
	41	B10_SSC	0.0338	3.18	6.70
	42	Average of 'B10_SSC'	0.0341	3.21	6.77
Mean			**0.0341**	**3.21**	**6.77**
1xStd Dev			**0.000264**	**0.0273**	**0.0686**
1xRSD (%)			**0.774**	**0.851**	**1.01**

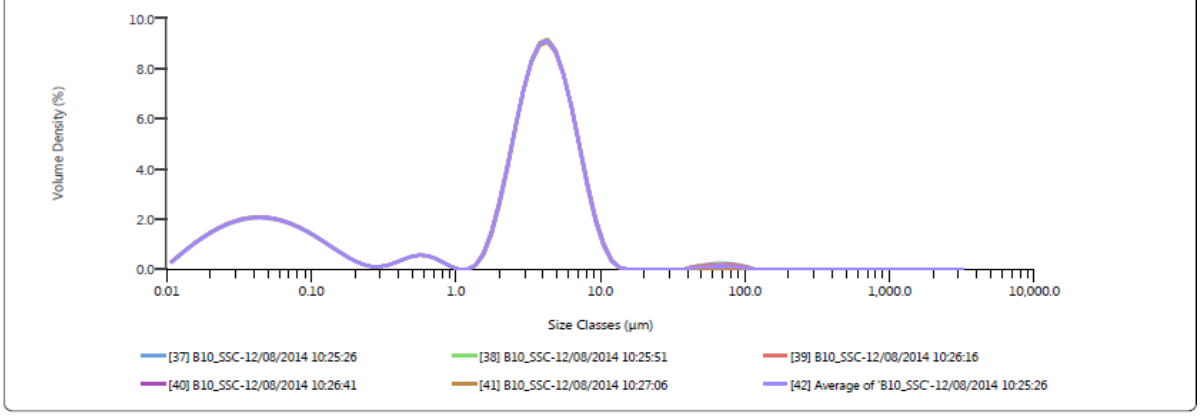
Analysis

Particle Name	Polycaprolacto	Particle Refractive Index	1.500
Dispersant Name	Water	Dispersant Refractive Index	1.330
Particle Absorption Index	0.001	Laser Obscuration	6.83 %
Weighted Residual	0.51 %	Scattering Model	Mie
Analysis Model	General Purpose	Analysis Sensitivity	Normal

Result

Concentration	0.0038 %	Span	2.105
Uniformity	0.917	Result Units	Volume
Specific Surface Area	46640 m²/kg	Dv (10)	0.0345 µm
D [3,2]	0.129 µm	Dv (50)	3.25 µm
D [4,3]	4.06 µm	Dv (90)	6.87 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.23	0.0679	1.50	0.460	0.43	3.12	6.95	21.2	0.00	144	0.00	976	0.00
0.0114	0.45	0.0771	1.38	0.523	0.48	3.55	7.55	24.1	0.00	163	0.00	1110	0.00
0.0129	0.66	0.0876	1.25	0.594	0.46	4.03	7.67	27.4	0.00	186	0.00	1260	0.00
0.0147	0.86	0.0995	1.10	0.675	0.37	4.58	7.31	31.1	0.00	211	0.00	1430	0.00
0.0167	1.04	0.113	0.93	0.767	0.23	5.21	6.52	35.3	0.00	240	0.00	1630	0.00
0.0189	1.21	0.128	0.76	0.872	0.09	5.92	5.38	40.1	0.10	272	0.00	1850	0.00
0.0215	1.36	0.146	0.58	0.991	0.00	6.72	4.04	45.6	0.14	310	0.00	2100	0.00
0.0244	1.48	0.166	0.42	1.13	0.00	7.64	2.72	51.8	0.18	352	0.00	2390	0.00
0.0278	1.58	0.188	0.28	1.28	0.10	8.68	1.58	58.9	0.21	400	0.00	2710	0.00
0.0315	1.65	0.214	0.17	1.45	0.45	9.86	0.75	66.9	0.22	454	0.00	3080	0.00
0.0358	1.69	0.243	0.10	1.65	1.12	11.2	0.25	76.0	0.20	516	0.00	3500	
0.0407	1.71	0.276	0.09	1.88	2.11	12.7	0.04	86.4	0.16	586	0.00		
0.0463	1.70	0.314	0.13	2.13	3.36	14.5	0.00	98.1	0.10	666	0.00		
0.0526	1.66	0.357	0.22	2.42	4.70	16.4	0.00	111	0.00	756	0.00		
0.0597	1.59	0.405	0.33	2.75	5.95	18.7	0.00	127	0.00	859	0.00		

Mastersizer PCL 10kDa : PVA 30-70kDa (+FLT)

Analysis

Created by: DT
Last edited: 04/03/2014 15:53:33

Malvern

Measurement Details

Sample Name	FLT-30-70-PVA-10k-PCL-B2a	Measurement Date Time	14/07/2014 11:39:58
Operator Name	DT	Analysis Date Time	14/07/2014 11:39:58
SOP File Name	HydroEV.cfg	Result Source	Measurement

	Record Number	Sample Name	Dx (10) (µm)	Dx (50) (µm)	Dx (90) (µm)
	13	FLT-30-70-PVA-10k-PCL-B2a	0.0334	3.35	7.45
	14	FLT-30-70-PVA-10k-PCL-B2a	0.0331	3.32	7.28
	15	FLT-30-70-PVA-10k-PCL-B2a	0.0330	3.31	7.21
	16	FLT-30-70-PVA-10k-PCL-B2a	0.0328	3.29	7.16
	17	FLT-30-70-PVA-10k-PCL-B2a	0.0327	3.28	7.13
	18	Average of 'FLT-30-70-PVA-10k-PCL-B2a'	0.0330	3.31	7.24
Mean			**0.0330**	**3.31**	**7.25**
1xStd Dev			**0.000230**	**0.0223**	**0.113**
1xRSD (%)			**0.696**	**0.673**	**1.57**

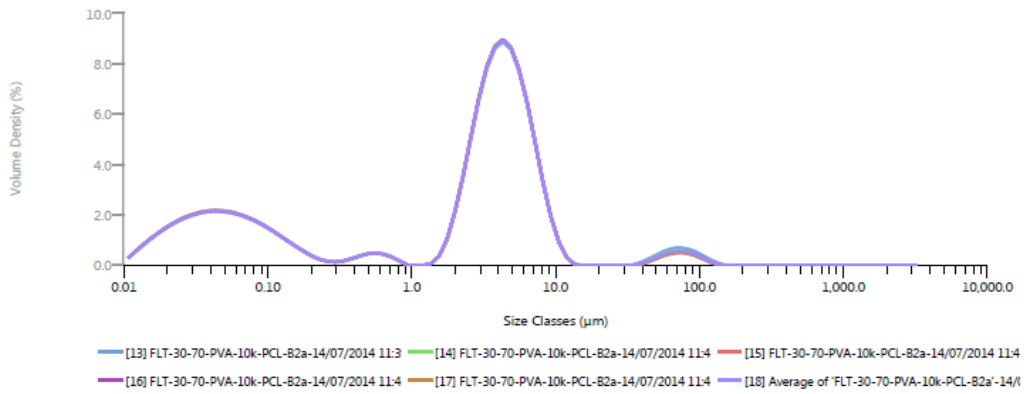
Analysis

Particle Name	Polycaprolacto	Particle Refractive Index	1.500
Dispersant Name	Water	Dispersant Refractive Index	1.330
Particle Absorption Index	0.001	Laser Obscuration	8.00 %
Weighted Residual	0.54 %	Scattering Model	Mie
Analysis Model	General Purpose	Analysis Sensitivity	Normal

Result

Concentration	0.0048 %	Span	2.215
Uniformity	1.447	Result Units	Volume
Specific Surface Area	48480 m²/kg	Dv (10)	0.0334 µm
D [3,2]	0.124 µm	Dv (50)	3.35 µm
D [4,3]	5.92 µm	Dv (90)	7.45 µm

Frequency (compatible)



Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In	Size (µm)	% Volume In
0.0100	0.24	0.0679	1.56	0.460	0.37	3.12	6.54	21.2	0.00	144	0.00	976	0.00
0.0114	0.47	0.0771	1.45	0.523	0.40	3.55	7.21	24.1	0.00	163	0.00	1110	0.00
0.0129	0.69	0.0876	1.31	0.594	0.37	4.03	7.43	27.4	0.00	186	0.00	1260	0.00
0.0147	0.90	0.0995	1.15	0.675	0.28	4.58	7.18	31.1	0.00	211	0.00	1430	0.00
0.0167	1.09	0.113	0.98	0.767	0.15	5.21	6.48	35.3	0.07	240	0.00	1630	0.00
0.0189	1.26	0.128	0.81	0.872	0.00	5.92	5.40	40.1	0.18	272	0.00	1850	0.00
0.0215	1.41	0.146	0.63	0.991	0.00	6.72	4.09	45.6	0.32	310	0.00	2100	0.00
0.0244	1.54	0.166	0.46	1.13	0.00	7.64	2.76	51.8	0.45	352	0.00	2390	0.00
0.0278	1.64	0.188	0.31	1.28	0.04	8.68	1.60	58.9	0.55	400	0.00	2710	0.00
0.0315	1.72	0.214	0.20	1.45	0.27	9.86	0.75	66.9	0.59	454	0.00	3080	0.00
0.0358	1.76	0.243	0.12	1.65	0.82	11.2	0.24	76.0	0.57	516	0.00	3500	
0.0407	1.78	0.276	0.10	1.88	1.72	12.7	0.03	86.4	0.47	586	0.00		
0.0463	1.77	0.314	0.14	2.13	2.91	14.5	0.00	98.1	0.34	666	0.00		
0.0526	1.73	0.357	0.21	2.42	4.23	16.4	0.00	111	0.19	756	0.00		
0.0597	1.66	0.405	0.30	2.75	5.50	18.7	0.00	127	0.08	859	0.00		

Appendix : 3.6.1.

Standard Calibration Curve of Flutamide Data

Stock Solution 5mg in 100ml of Ethanol	Absorbance at 300nm **Sample 1**		Actual Concentration **(mg/ml)**	Absorbance at 300nm **Sample 2**		Actual Concentration **(mg/ml)**	Absorbance at 300nm **Sample 3**
15	0.003		0.000078125	0.002		0.000078125	0.007
14	0.009		0.00015625	0.008		0.00015625	0.005
13	0.064		0.00003125	0.068		0.00003125	0.065
12	0.074		0.000625	0.073		0.000625	0.073
11	0.087		0.00125	0.084		0.00125	0.081
10	0.103		0.0025	0.103		0.0025	0.101
9	0.211		0.005	0.215		0.005	0.218
8	0.427		0.01	0.420		0.01	0.317
7	0.504		0.015	0.605		0.015	0.635
6	0.871		0.02	0.778		0.02	0.719
5	0.915		0.025	0.926		0.025	0.985
4	1.150		0.03	1.078		0.03	1.088
3	1.330		0.035	1.276		0.035	1.337
2	1.544		0.04	1.469		0.04	1.518
1	1.725		0.045	1.704		0.045	1.718
Stock	1.885		Stock	1.905		Stock	1.883

Actual Conc (mg/ml)	**Average Absorbance at 300nm of all 3 samples**
0.000078125	0.00400
0.00015625	0.00733
0.00003125	0.06567
0.000625	0.07333
0.00125	0.08400
0.0025	0.10233
0.005	0.21467
0.01	0.38800
0.015	0.58133
0.02	0.78933
0.025	0.94200
0.03	1.10533
0.035	1.31433
0.04	1.51033
0.045	1.71567
0.05	1.89400

Appendix : 3.6.2.

In-vitro Percentage Drug Release (PDR) calculations

First calculate the theoretical 100% of FLT present in the 40mg (0.040g) sample used within the dissolution test:

The original sample yielded 400mg (0.4000g) PCL+FLT. Therefore:

(0.0400g/0.4000g)*100 = **10.00%** (20mg) of the PCL+FLT sample was used in the dissolution test.

Using this we can find out the theoretical 100% of FLT that was present in the 20mg sample used within the dissolution test:

(0.0500g/100)*10.00% = **5.000mg (0.005000g)**

This therefore shows that in the 20mg sample 8.000mg of FLT was encapsulated.

We can therefore calculate the concentration of the encapsulated FLT as:

5.000mg/900mL = **0.00555mg/mL**

Using the equation of the line from the standard curve we can calculate the concentration of FLT released at each time point:

e.g.: y=37.879x

x – Concentration (mg/mL)

y – Absorbance (300nm)

Therefore to find our concentration we use:

x = y/37.879 (all results as seen on the excel document).

Once the concentrations have been calculated we can calculate the Percentage Drug Released (PDR) as follows:

PDR = (concentration from time point obtained/initial concentration)*100

Appendix : 3.6.3.

Release Study Raw Data

PCL 80kDa/ PVA 13-23kDa			
Hours	**Absorbance (300nm)**	**Average Absorbance (300nm)**	**Standard Deviation (+/-)**
0	0	0	0
	0		
	0		
0.5	0.02	0.016333333	0.005507571
	0.01		
	0.019		
1	0.009	0.008666667	0.001527525
	0.01		
	0.007		
2	0.009	0.008333333	0.00305505
	0.011		
	0.005		
4	0.007	0.007333333	0.00057735
	0.007		
	0.008		
8	0.018	0.017666667	0.003511885
	0.014		
	0.021		
24	0.014	0.017	0.002645751
	0.018		
	0.019		
48	0.022	0.018666667	0.004932883
	0.021		
	0.013		
72	0.013	0.011	0.002645751
	0.012		
	0.008		
96	0.012	0.010333333	0.001527525
	0.01		
	0.009		
168	0.013	0.010666667	0.002516611

	0.011		
	0.008		
216	0.008	0.010333333	0.00321455
	0.014		
	0.009		
264	0.006	0.008333333	0.002516611
	0.011		
	0.008		
336	0.006	0.006333333	0.001527525
	0.008		
	0.005		
384	0	0.000666667	0.001154701
	0.002		
	0		
432	0	0	0
	0		
	0		

PCL 80kDa/ PVA 30-70kDa			
Hours	**Absorbance (300nm)**	**Average Absorbance (300nm)**	**Standard Deviation (+/-)**
0	0	0	0
	0		
	0		
0.5	0	0	0
	0		
	0		
1	0.01	0.012333333	0.002081666
	0.013		
	0.014		
2	0.006	0.009666667	0.00321455
	0.012		
	0.011		
4	0.008	0.010333333	0.00321455
	0.014		
	0.009		
8	0.012	0.011	0.001
	0.011		
	0.01		

24	0.016	0.014333333	0.001527525
	0.014		
	0.013		
48	0.02	0.022	0.002645751
	0.025		
	0.021		
72	0.022	0.019333333	0.002516611
	0.019		
	0.017		
96	0.011	0.012333333	0.006110101
	0.019		
	0.007		
168	0.013	0.009666667	0.002886751
	0.008		
	0.008		
216	0.019	0.019333333	0.00057735
	0.019		
	0.02		
264	0.011	0.014	0.002645751
	0.015		
	0.016		
336	0.009	0.009333333	0.001527525
	0.011		
	0.008		
384	0.005	0.005	0.003
	0.008		
	0.002		
432	0	0	0
	0		
	0		

PCL 65kDa/ PVA 13-23kDa			
Hours	**Absorbance (300nm)**	**Average Absorbance (300nm)**	**Standard Deviation (+/-)**
0	0	0	0
	0		
	0		
0.5	0.019	0.016333333	0.00305505
	0.013		
	0.017		
1	0.011	0.005333333	0.005131601
	0.004		
	0.001		
2	0.01	0.009666667	0.00057735
	0.009		
	0.01		
4	0.012	0.012333333	0.001527525
	0.011		
	0.014		
8	0.017	0.017	0.002
	0.015		
	0.019		
24	0.018	0.016666667	0.001527525
	0.015		
	0.017		
48	0.014	0.013	0.001
	0.013		
	0.012		
72	0.01	0.009666667	0.00057735
	0.01		
	0.009		
96	0.016	0.013	0.002645751
	0.012		
	0.011		
168	0.009	0.008666667	0.001527525
	0.01		
	0.007		
216	0.004	0.002666667	0.001527525
	0.001		
	0.003		

264	0.002	0.001666667	0.00057735
	0.002		
	0.001		
336	0	0	0
	0		
	0		
384	0	0	0
	0		
	0		
432	0	0	0
	0		
	0		

PCL 65kDa/ PVA 30-70kDa			
Hours	**Absorbance (300nm)**	**Average Absorbance (300nm)**	**Standard Deviation (+/-)**
0	0	0	0
	0		
	0		
0.5	0	0	0
	0		
	0		
1	0.006	0.007333333	0.001527525
	0.007		
	0.009		
2	0.007	0.009333333	0.002081666
	0.011		
	0.01		
4	0.007	0.008333333	0.00321455
	0.006		
	0.012		
8	0.011	0.011	0.003
	0.008		
	0.014		
24	0.019	0.019333333	0.001527525
	0.018		
	0.021		
48	0.02	0.020333333	0.00057735
	0.02		

	0.021		
72	0.011	0.014	0.003605551
	0.018		
	0.013		
96	0.01	0.011666667	0.002081666
	0.014		
	0.011		
168	0.009	0.010666667	0.003785939
	0.015		
	0.008		
216	0.006	0.01	0.003464102
	0.012		
	0.012		
264	0.004	0.006333333	0.002081666
	0.007		
	0.008		
336	0.004	0.005	0.001
	0.006		
	0.005		
384	0	0	0
	0		
	0		
432	0	0	0
	0		
	0		

PCL 10kDa/ PVA 13-23kDa			
Hours	**Absorbance (300nm)**	**Average Absorbance (300nm)**	**Standard Deviation (+/-)**
0	0	0	0
	0		
	0		
0.5	0.007	0.007333333	0.001527525
	0.006		
	0.009		
1	0.009	0.008666667	0.001527525
	0.007		
	0.01		
2	0.01	0.009666667	0.001527525
	0.011		
	0.008		
4	0.008	0.007	0.001732051
	0.005		
	0.008		
8	0.006	0.008	0.002
	0.008		
	0.01		
24	0.011	0.009666667	0.001527525
	0.01		
	0.008		
48	0.006	0.007	0.001
	0.007		
	0.008		
72	0.005	0.006333333	0.001527525
	0.008		
	0.006		
96	0.002	0.002	0.001
	0.003		
	0.001		
168	0	0	0
	0		
	0		
216	0	0	0
	0		
	0		

264	0	0	0
	0		
	0		
336	0	0	0
	0		
	0		
384	0	0	0
	0		
	0		
432	0	0	0
	0		
	0		

PCL 10kDa/ PVA 30-70kDa			
Hours	**Absorbance (300nm)**	**Average Absorbance (300nm)**	**Standard Deviation (+/-)**
0	0	0	0
	0		
	0		
0.5	0.005	0.004333333	0.004041452
	0		
	0.008		
1	0.006	0.008	0.002645751
	0.011		
	0.007		
2	0.003	0.006666667	0.003511885
	0.007		
	0.01		
4	0.008	0.009	0.001732051
	0.008		
	0.011		
8	0.012	0.012	0.001
	0.013		
	0.011		
24	0.012	0.011666667	0.002516611
	0.014		
	0.009		
48	0.009	0.007	0.003464102

	0.003		
	0.009		
72	0.006	0.003666667	0.00321455
	0.005		
	0		
96	0.006	0.003666667	0.002516611
	0.004		
	0.001		
168	0.001	0.003	0.002
	0.003		
	0.005		
216	0	0	0
	0		
	0		
264	0	0	0
	0		
	0		
336	0	0	0
	0		
	0		
384	0	0	0
	0		
	0		
432	0	0	0
	0		
	0		

Appendix : 3.6.4.

Drug Release Profile Calculation Table

PCL 80kDa/ PVA 13-23kDa							
Hours	**Average Absorbance**	**Cumulative Absorbance**	**x=y/37.879 - Concentration (mg/ml)**	**Percentage Drug Release (%)**	**Standard Deviation**	**Standard Dev (Concentration)**	**Standard Dev (Percentage)**
0	0	0	0	0	0	0	0
0.5	0.016333333	0.016333333	0.000431198	7.769325861	0.005507571	0.000145399	2.619802671
1	0.008666667	0.025	0.000659996	11.8918253	0.001527525	4.03264E-05	0.726602528
2	0.008333333	0.033333333	0.000879995	15.85576706	0.00305505	8.06529E-05	1.453205055
4	0.007333333	0.040666667	0.001073594	19.34403582	0.00057735	1.5242E-05	0.274629941
8	0.017666667	0.058333333	0.001539991	27.74759236	0.003511885	9.27132E-05	1.670508718
24	0.017	0.075333333	0.001988789	35.83403356	0.002645751	6.98474E-05	1.258512495
48	0.018666667	0.094	0.002481586	44.71326312	0.004932883	0.000130227	2.346439249
72	0.011	0.105	0.002771984	49.94566625	0.002645751	6.98474E-05	1.258512495
96	0.010333333	0.115333333	0.003044783	54.86095404	0.001527525	4.03264E-05	0.726602528
168	0.010666667	0.126	0.003326381	59.9347995	0.002516611	6.64382E-05	1.197084162
216	0.010333333	0.136333333	0.00359918	64.85008729	0.00321455	8.48637E-05	1.529074801
264	0.008333333	0.144666667	0.003819179	68.81402906	0.002516611	6.64382E-05	1.197084162
336	0.006333333	0.151	0.003986378	71.8266248	0.001527525	4.03264E-05	0.726602528
384	0.000666667	0.151666667	0.004003978	72.14374014	0.001154701	3.04839E-05	0.549259883
432	0	0.151666667	0.004003978	72.14374014	0	0	0

PCL 80kDa/ PVA 30-70kDa							
Hours	**Average Absorbance**	**Cumulative Absorbance**	**x=y/37.879 - Concentration (mg/ml)**	**Percentage Drug Release (%)**	**Standard Deviation**	**Standard Dev (Concentration)**	**Standard Dev (Percentage)**
0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0
1	0.012333333	0.012333333	0.000325598	5.866633814	0.002081666	5.49557E-05	0.990192336
2	0.009666667	0.022	0.000580797	10.46480626	0.00321455	8.48637E-05	1.529074801
4	0.010333333	0.032333333	0.000853595	15.38009405	0.00321455	8.48637E-05	1.529074801
8	0.011	0.043333333	0.001143994	20.61249718	0.001	2.63999E-05	0.475673012
24	0.014333333	0.057666667	0.001522391	27.43047702	0.001527525	4.03264E-05	0.726602528
48	0.022	0.079666667	0.002103188	37.89528328	0.002645751	6.98474E-05	1.258512495
72	0.019333333	0.099	0.002613585	47.09162818	0.002516611	6.64382E-05	1.197084162
96	0.012333333	0.111333333	0.002939184	52.95826199	0.006110101	0.000161306	2.906410111
168	0.009666667	0.121	0.003194382	57.55643444	0.002886751	7.62098E-05	1.373149707
216	0.019333333	0.140333333	0.003704779	66.75277934	0.00057735	1.5242E-05	0.274629941
264	0.014	0.154333333	0.004074377	73.4122015	0.002645751	6.98474E-05	1.258512495
336	0.009333333	0.163666667	0.004320776	77.85181628	0.001527525	4.03264E-05	0.726602528
384	0.005	0.168666667	0.004452775	80.23018134	0.003	7.91996E-05	1.427019036
432	0	0.168666667	0.004452775	80.23018134	0	0	0

PCL 65kDa/ PVA 13-23kDa							
Hours	**Average Absorbance**	**Cumulative Absorbance**	**x=y/37.879 - Concentration (mg/ml)**	**Percentage Drug Release (%)**	**Standard Deviation**	**Standard Dev (Concentration)**	**Standard Dev (Percentage)**
0	0	0	0	0	0	0	0
0.5	0.016333333	0.016333333	0.000431198	7.769325861	0.00305505	8.06529E-05	1.453205055
1	0.005333333	0.021666667	0.000571997	10.30624859	0.005131601	0.000135474	2.440964313
2	0.009666667	0.031333333	0.000827195	14.90442104	0.00057735	1.5242E-05	0.274629941
4	0.012333333	0.043666667	0.001152794	20.77105485	0.001527525	4.03264E-05	0.726602528
8	0.017	0.060666667	0.001601591	28.85749606	0.002	5.27997E-05	0.951346024
24	0.016666667	0.077333333	0.002041589	36.78537959	0.001527525	4.03264E-05	0.726602528
48	0.013	0.090333333	0.002384787	42.96912874	0.001	2.63999E-05	0.475673012
72	0.009666667	0.1	0.002639985	47.56730119	0.00057735	1.5242E-05	0.274629941
96	0.013	0.113	0.002983183	53.75105035	0.002645751	6.98474E-05	1.258512495
168	0.008666667	0.121666667	0.003211982	57.87354978	0.001527525	4.03264E-05	0.726602528
216	0.002666667	0.124333333	0.003282382	59.14201115	0.001527525	4.03264E-05	0.726602528
264	0.001666667	0.126	0.003326381	59.9347995	0.00057735	1.5242E-05	0.274629941
336	0	0.126	0.003326381	59.9347995	0	0	0
384	0	0.126	0.003326381	59.9347995	0	0	0
432	0	0.126	0.003326381	59.9347995	0	0	0

PCL 65kDa/ PVA 30-70kDa							
Hours	**Average Absorbance**	**Cumulative Absorbance**	**x=y/37.879 - Concentration (mg/ml)**	**Percentage Drug Release (%)**	**Standard Deviation**	**Standard Dev (Concentration)**	**Standard Dev (Percentage)**
0	0	0	0	0	0	0	0
0.5	0	0	0	0	0	0	0
1	0.007333333	0.007333333	0.000193599	3.488268754	0.001527525	4.03264E-05	0.726602528
2	0.009333333	0.016666667	0.000439998	7.927883532	0.002081666	5.49557E-05	0.990192336
4	0.008333333	0.025	0.000659996	11.8918253	0.00321455	8.48637E-05	1.529074801
8	0.011	0.036	0.000950395	17.12422843	0.003	7.91996E-05	1.427019036
24	0.019333333	0.055333333	0.001460792	26.32057333	0.001527525	4.03264E-05	0.726602528
48	0.020333333	0.075666667	0.001997589	35.99259123	0.00057735	1.5242E-05	0.274629941
72	0.014	0.089666667	0.002367187	42.6520134	0.003605551	9.5186E-05	1.715063435
96	0.011666667	0.101333333	0.002675185	48.20153187	0.002081666	5.49557E-05	0.990192336
168	0.010666667	0.112	0.002956783	53.27537733	0.003785939	9.99482E-05	1.800868958
216	0.01	0.122	0.003220782	58.03210745	0.003464102	9.14518E-05	1.647779649
264	0.006333333	0.128333333	0.003387981	61.04470319	0.002081666	5.49557E-05	0.990192336
336	0.005	0.133333333	0.00351998	63.42306825	0.001	2.63999E-05	0.475673012
384	0	0.133333333	0.00351998	63.42306825	0	0	0
432	0	0.133333333	0.00351998	63.42306825	0	0	0

PCL 10kDa/ PVA 13-23kDa							
Hours	**Average Absorbance**	**Cumulative Absorbance**	**x=y/37.879 - Concentration (mg/ml)**	**Percentage Drug Release (%)**	**Standard Deviation**	**Standard Dev (Concentration)**	**Standard Dev (Percentage)**
0	0	0	0	0	0	0	0
0.5	0.007333333	0.007333333	0.000193599	3.488268754	0.001527525	4.03264E-05	0.726602528
1	0.008666667	0.016	0.000422398	7.610768191	0.001527525	4.03264E-05	0.726602528
2	0.009666667	0.025666667	0.000677596	12.20894064	0.001527525	4.03264E-05	0.726602528
4	0.007	0.032666667	0.000862395	15.53865172	0.001732051	4.57259E-05	0.823889824
8	0.008	0.040666667	0.001073594	19.34403582	0.002	5.27997E-05	0.951346024
24	0.009666667	0.050333333	0.001328793	23.94220827	0.001527525	4.03264E-05	0.726602528
48	0.007	0.057333333	0.001513592	27.27191935	0.001	2.63999E-05	0.475673012
72	0.006333333	0.063666667	0.001680791	30.28451509	0.001527525	4.03264E-05	0.726602528
96	0.002	0.065666667	0.00173359	31.23586112	0.001	2.63999E-05	0.475673012
168	0	0.065666667	0.00173359	31.23586112	0	0	0
216	0	0.065666667	0.00173359	31.23586112	0	0	0
264	0	0.065666667	0.00173359	31.23586112	0	0	0
336	0	0.065666667	0.00173359	31.23586112	0	0	0
384	0	0.065666667	0.00173359	31.23586112	0	0	0
432	0	0.065666667	0.00173359	31.23586112	0	0	0

PCL 10kDa/ PVA 30-70kDa							
Hours	**Average Absorbance**	**Cumulative Absorbance**	**x=y/37.879 - Concentration (mg/ml)**	**Percentage Drug Release (%)**	**Standard Deviation**	**Standard Dev (Concentration)**	**Standard Dev (Percentage)**
0	0	0	0	0	0	0	0
0.5	0.004333333	0.004333333	0.000114399	2.061249718	0.004041452	0.000106694	1.92240959
1	0.008	0.012333333	0.000325598	5.866633814	0.002645751	6.98474E-05	1.258512495
2	0.006666667	0.019	0.000501597	9.037787226	0.003511885	9.27132E-05	1.670508718
4	0.009	0.028	0.000739196	13.31884433	0.001732051	4.57259E-05	0.823889824
8	0.012	0.04	0.001055994	19.02692048	0.001	2.63999E-05	0.475673012
24	0.011666667	0.051666667	0.001363992	24.57643895	0.002516611	6.64382E-05	1.197084162
48	0.007	0.058666667	0.001548791	27.90615003	0.003464102	9.14518E-05	1.647779649
72	0.003666667	0.062333333	0.001645591	29.65028441	0.00321455	8.48637E-05	1.529074801
96	0.003666667	0.066	0.00174239	31.39441879	0.002516611	6.64382E-05	1.197084162
168	0.003	0.069	0.00182159	32.82143782	0.002	5.27997E-05	0.951346024
216	0	0.069	0.00182159	32.82143782	0	0	0
264	0	0.069	0.00182159	32.82143782	0	0	0
336	0	0.069	0.00182159	32.82143782	0	0	0
384	0	0.069	0.00182159	32.82143782	0	0	0
432	0	0.069	0.00182159	32.82143782	0	0	0

Appendix : 5.2.1.

Task Name: Cytotoxicity Test

Date:

Location: Cell Culture Research Lab (Keith HoldingK.Holding@wlv.ac.uk Ext 2655 and Dr. Angie Williams A.S.Williams@wlv.ac.uk Ext 2128)

1. Chemicals

- Cancer cell lines
- *Eagle's Minimal Essential Medium* + 2mM Glutamine + 1% Non-Essential Amino Acids + 10% Foetal Bovine Serum. (DMEM)
- *Phosphate-Buffered Saline (PBS)*
- (Trypsin)-EDTA solution
- Thiazolyl Blue Tetrazolium Bromide (MTT)
- Dimethyl Sulfoxide(DMSO)
- Sorensen's buffer (Sodium Chloride + Glycine)

2. Glassware, facilities and instruments

- Microtitre plate reader (540 nm)
- 96-well Microtitre plate (flat-bottomed)
- Inverted microscope
- Multi-channel pipette
- CO_2 controlled incubator
- Laminar flow hood
- Sterile tubes (5 mL)
- Pasteur pipettes
- Sterile pipette tips
- Conical centrifuge tube
- Centrifuge – 1500 x *g*
- T75 Culture flask (Filtered cap)
- Haemocytometer
- Haemocytometer cover slip
- Magnetic Stirrer
- Hot Plate
- 0.45 µm Plastic vacuum filter (Vol. 500 mL)
- Foil
- 750 mL beaker

- Waste Container

3. Protocol

Experimental Day 1

Sub-culturing

1. Visually check the tissue culture medium for contamination or cellular deterioration. **(See Note 1)**
2. Examine adherent cells for 70-80% confluence using an inverted light microscope.
3. Aspirate the spent media using a sterile Pasteur pipette from the culture flask into a waste container.
4. Measure out 5ml of *Phosphate-Buffered Saline (PBS)*.
5. Wash adherent cells in 5ml of *PBS*.
6. Aspirate the solution using a sterile pipette into a waste container.
7. Measure out 2.0 to 3.0 mL of Trypsin-EDTA solution.
8. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to culture flask.
9. Incubate the culture flask for 2-3 minutes at 37°C.
10. Observe cells under an inverted microscope until cell layer is detached.
11. Measure out 3.0 to 5.0mL of Dulbecco's Modified Eagle medium (DMEM).
12. Add 3 to 5 ml of DMEM into the culture flask.
13. Transfer the cell suspension into a conical centrifuge tube.
14. Place the conical centrifuge tube into a centrifuge and spin cells at 1300-1500 rpm for 5 minutes.
15. Aspirate the supernatant into a waste container, leaving the pellet.
16. Measure out 8 to 10mL of DMEM depending on the size of the pellet.
17. Re-suspend the pellet in 6-8 mL of DMEM into the culture flask.
18. Gently pipette to ensure a homogenous solution of single cells.
19. Pipette 10µL of the cell suspension onto a haemocytometer with haemocytometer cover slip (it is a special cover slip you can get from sigma) and examine the cells under the inverted light microscope to see if the cells are present.**(See Note 2)**
20. Count the cells using a counter – 1 cell is equal to 10,000 cells/mL of the suspension you have.

21. Calculate how many wells you have and how much cells you need per well.

    - **Example**: You have 3 plates with 60 working wells in each (leaving outside wells for water) the total nº of wells is 180.
    - 180 wells x 200 µl = 36,000 µl = 36 mL. So you can prepare **40 mL.**
    - At the same time if you need 5,000 cells in each well / 200µl.
    - Therefore; 1 mL/25,000 cells.
    - 10 mL/ 250,000 cells or $0.25x10^6$ cells.
    - For 40 mL it will be $1x10^6$ cells.

    - If you have $0.5x10^6$ cells/ mL in your actual cell suspension after counting.
    - Add 2mL of Cells + 38 mL of fresh DMEM to make $1x10^6$ cells/40 ml.
    - In other words 5,000 cells/ 200 µL

    - Mix cells and media well to make uniform suspension for accuracy.

22. Use a petri dish or multichannel pipette dish and pour your calculated cell suspension and aliquot 200 µL in each well using a multi-channel pipette. For accuracy frequent mixing in between is essential.

23. Incubate the 96 well plates for 24 h at 37°C, 5% CO_2 (CO_2 controlled incubator).

Experimental Day 2

1. Aspirate off the media without disturbing the layer of cells using a Pasteur pipette.

2. Calculate the concentration of the initial test drug for 1:2 serial dilutions.

    - **Example**: Temozolomide (TmZ) needed at an initial concentration of 500 µM in 8ml PBS solution.

    - 8 mL = 8000 µL
    - 100 mM Stock TmZ = 100,000 µM Stock TmZ

    - $\frac{8000\ \mu L \times 500\ \mu M}{100{,}000\ \mu M}$ = **40 µL** TmZ

    - Therefore; 40 µL TmZ + 7,960 40 µL PBS = 8000 µL (TmZ 500 µM in 8ml PBS solution)

3. Using 8 test tubes pour your calculated measure of the test drug and PBS into the first test tube to make an 8mL solution (Test tube 1).

4. Measure out 30mL of PBS into a beaker using a pipette.

5. Aliquot 4mL of PBS into each of the 7 remaining test tubes (Test Tubes 2 - 8).

6. Transfer 4 mL of Test Tube 1 solution into Test tube 2 using a pipette.

7. Repeat this procedure transferring 4 mL of solution from test tubes 2 to 3, 3 to 4, 4 to 5, 5 to 6, 6 to 7 and 7 to 8. (High to Low Concentration).

8. Aliquot 200 µL of each dilution into the allocated wells in the three plates.

9. Incubate the plate for an appropriate length of time at 37°C (5% CO_2) subject to the drug used.

Experimental Day 3

1) Pippette 20 µL of MTT solution into each well of the cell plate.

2) Place 96-well plate lid over the plate and wrap with foil, then incubate (37°C, 5% CO_2) for 4 hours. **(See Note 3)**

3) Aspirate the media from the wells.

4) Pipette 80 µL of Dimethyl Sulfoxide (DMSO)into each well.

5) Pipette 20 µL of Sorensen's buffer into each well.

6) Read on a multi-well plate reader at 540 nm.

4. Preparations

MTT Stock Solution

1) Weigh out 2.5g of MTT stock.

2) Measure out 500 mL PBS in 750 mL beaker.

3) Dissolve MTT stock in PBS under gentle stirring (6-8 hrs) and wrap the beaker with foil.

4) Filter the MTT solution using a 0.45 µm Plastic vacuum filter (Vol. 500 mL) and wrap with foil (Store at 2-4^{o}C).**(See Note 3)**

Sorensen's Buffer

1) Weigh out 2.9g of Sodium Chloride.

2) Weigh out 3.8g of Glycine.

3) Dissolve the Sodium Chloride and Glycine together in 500 mL distilled water.

5. Notes to highlight

Note1: Check for cell medium contamination – DMEM solution will turn from pink/ red to orange showing a change in pH due to CO_2 absorption.

Note 2: Check for clumping of cells - if any clumps are present re-suspend and follow procedure from step 14.

Note 3: Cover MTT solution with foil as it is light sensitive to prevent photo degradation. Solution must be stored at 2-4°C in the dark (Up to 18months).

4. Precautions

MTT is toxic and may cause heritable genetic defects. In case of contact, immediately flush eyes or skin with copious amounts of water. If swallowed, wash out mouth with water provided person is conscious. Call a physician.

5. References

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